

# SOIL SCIENCE

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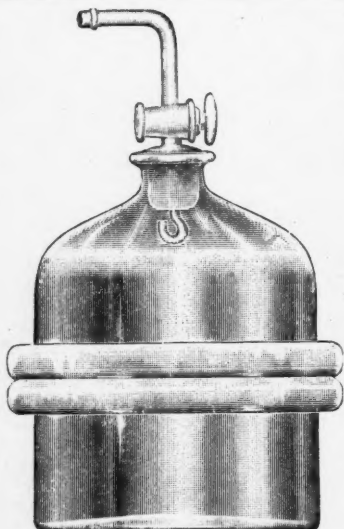
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## PARTIAL STERILIZATION OF SOIL, MICROBIOLOGICAL ACTIVITIES AND SOIL FERTILITY: I<sup>1</sup>

SELMAN A. WAKSMAN AND ROBERT L. STARKEY

*New Jersey Agricultural Experiment Stations*

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The soil harbors a large number of microorganisms belonging to various groups which have different physiological functions. Conditions favorable to some organisms may prove injurious to others. The various bacteria, algae, fungi, actinomycetes, protozoa, nematodes, etc., carry on their activities which may or may not lead to phenomena favorable to soil fertility; some decompose organic matter with the liberation of ammonia, some use the nitrogen-free organic matter as a source of energy to fix atmospheric nitrogen, while others assimilate the ammonia using the carbohydrate as a source of energy and build up microbial proteins; still others use the ammonia as a source of energy and convert it into nitrites and then nitrates. Some organisms produce substances toxic or beneficial to others, while some actually consume others. All these various groups of organisms are united by the one common medium—the soil. As a medium the soil is affected by the content of nutrients, the condition in which these nutrients are present in the soil, the water content of the soil and transportation of salts, soil aeration, oxygen tension, carbon dioxide production, soil temperature and colloidal structure of the soil, with surface and adsorption phenomena.

When any one of these factors is changed, there is a corresponding change in the biological composition of the soil, in other words any change of the physical, physico-chemical and chemical soil condition will greatly modify the biological flora. The extent of this modification is, however, only partly known. We know, for example, that a change in soil reaction will bring about a relative change in the number of microorganisms. The same is true of soil aeration or lack of aeration. The addition of undecomposed organic matter will greatly stimulate the development of various microorganisms. Partial sterilization of the soil has been extensively studied during the last few years. This was due to the stimulus given by the excellent contributions of Russell and his associates and the hypothesis put forth on the destructive influence of protozoa upon bacteria. The limitation of the activities of soil bacteria is supposed to result in a limitation of soil fertility. According to Russell, the partial sterilization of soil by volatile antiseptics or steam brings about a

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destruction of the protozoa; this stimulates the development of bacteria and increases the fertility of the soil. This hypothesis has been criticized by different investigators who found that the favorable influence of heat and antiseptics upon the soil flora and growth of cultivated plants is due to other phenomena, like the destruction of pathogenic organisms, direct stimulation of plants, liberation of available plant-food, dissolution or redistribution of the soil fats, destruction of toxins, etc.

Without reviewing in detail the subject of partial sterilization of soil, which has been done by Kopeloff and associates (29), we may merely point out some of the facts that have been established as bearing upon the problem at hand. The purpose of the following investigations is not to disprove or to prove any fixed theory, but to throw light on the interrelationships of soil microorganisms and their activities as affected by various soil treatments, including partial sterilization.

The established facts in regard to the influence of partial sterilization upon soil biological activities may be briefly summarized as follows:

1. Partial sterilization of soil by heating above 60°C. or by treatment with vapors of volatile antiseptics such as toluene and carbon bisulfide, causes a fall and then a rapid rise in bacterial numbers. The resulting numbers of bacteria are far above the numbers in the untreated soil [Hiltner and Störmer (21), Russell and Hutchinson (47, 48), Fred (11, 12)].

2. The rise in numbers of bacteria is accompanied by an increase in the accumulation of ammonia over the untreated soil; this is formed from the decomposition of the organic nitrogen compounds in the soil [Störmer (51, 52), Russell and Hutchinson (47, 48), Scherpe (49), Fred (11, 12)].

3. When untreated soil is added to partially sterilized soil, there is a more rapid increase of bacterial numbers and ammonia accumulation than in the uninoculated soil. The initial rise in the former soil is followed sooner by a fall in numbers than in the uninoculated, especially in soils rich in organic matter [Russell (46), Fred (12)].

4. In the uninoculated soil, bacterial numbers remain at a high level for a long time [Hiltner and Störmer (21), Russell and Hutchinson (47, 48)].

5. There is some evidence for the assumption that the rapid bacterial development may be due to the elimination of other organisms, originally present in the soil, ("detrimental factor" according to Russell and Hutchinson) by the use of heat or antiseptics.

6. Protozoa, particularly the amoebae and ciliates are killed in the process of partial sterilization [Russell and Hutchinson (47, 48), Buddin (4)]. Since certain protozoa, particularly ciliates and amoebae are known to destroy bacteria in liquid cultures [see also Cutler (6)], Russell and Hutchinson assumed that the protozoa are the detrimental organisms, which keep in check bacterial development. This assumption was further substantiated by the demonstration of Cutler, Crump and Sandon (7) that an inverse relationship was found between the numbers of bacteria and active amoebae in 86 per cent of the observations made by counting these organisms on 365 consecutive days.

7. In addition to bacteria, other groups of soil microorganisms, particularly the fungi and actinomycetes are capable of decomposing soil organic matter with the liberation of ammonia.

8. Heat and volatile antiseptics have a destructive influence upon soil fungi [Bolley (2), Waget (55)].

9. Nothing has been said in the work of Russell and associates about the rôle of fungi in the soil. There may be hundreds of thousands of these organisms in a gram of soil, especially in soils rich in organic matter, both in the form of spores and as vegetative mycelium.

Moore (35) pointed out that, "to those that are familiar with the rapidity and certainty with which some of the fungi reduce organic to inorganic matter it is evident that there is no group of organisms present in the soil more capable of producing profound changes in its environment."

10. Fungi utilize available energy very economically, converting up to 50 per cent of the available carbon into fungous mycelium [Kruse (31)]. Since fungous mycelium contains 3 to 7 per cent of nitrogen, large quantities of nitrogen have to be assimilated in the synthesis of fungous protoplasm. Bacteria make only a limited growth in comparison with the amount of change produced, and will, therefore, convert only comparatively small amounts of nitrogen into microbial protein.

11. The actinomycetes, which form 10 to 50 per cent of the organisms developing on the plate, grow only slowly, particularly in soil treated with antiseptics [Hiltner and Störmer (21), Greig-Smith (18)].

12. The bacteria that account for the rapid rise in numbers after partial sterilization belong chiefly to the slow-growing, non-spore forming, gelatin non-liquefying bacteria [Hiltner and Störmer (21)]. There is some evidence that under certain conditions, at least, spore-bearing (butyric acid) bacteria develop rapidly [Truffaut and Bezsonoff (53)].

13. Nitrifying bacteria are destroyed or temporarily put out of action by partial sterilization, but when reintroduced they become very active [Störmer (51, 52), Russell and Hutchinson (47, 48), Fred (11, 12)].

14. Protozoa, at least most flagellates and some ciliates, can also live in organic and inorganic media [Peters (41), Aliekseiev (1)], without depending for their nutrients upon living bacteria. It is even possible that the protozoa may play a decidedly beneficial rôle in the transformation of organic and inorganic substances in the soil, as we might conclude from the results of Crabtree (5) on the protozoa in the sewage bed and Aliekseiev (1) on the protozoa in the manure pile. Protozoa may also feed on smaller protozoa and on fungous mycelium [Francé (10)], while Goodey (15) found that the addition of protozoa to a soil free from these organisms did not act as a factor limiting bacterial activities. According to Nasir (38) protozoa exert no depressing effect on nitrogen-fixing bacteria, both in sand and in solution; as a matter of fact the presence of protozoa resulted in a decided increase in nitrogen-fixation in 31 out of 36 experiments.

15. Not all the protozoa, especially the flagellates, are always destroyed by the disinfectant [Gainey (13, 14), Fred (11, 12), also Greig-Smith].

Changes in the microbial flora of the soil similar to those produced by partial sterilization will result also from other treatments of soil.

16. Air-drying of soil followed by moistening also produced a decidedly stimulating effect upon bacterial development [Rahn (44), Ritter (45)].

17. Calcium oxide produces a condition in the soil very similar to partial sterilization [Miller (34), Hutchinson (22), Hutchinson and McLennan (23)].

18. Treatment of soil with lime, antiseptics and heat makes soluble a great deal of plant food, including carbon and nitrogen compounds, as well as phosphates and other minerals. This was shown by Hutchinson and McLennan (23) for lime; by Krüger and Schneidewind (30), Kelley (25), Boyoucos (3), Wilson (59), Fischer (9), Mann, *et al.* (33), and others for heat; and by Heinze (19, 20), Pickering (42) and others for disinfectants.

19. Antiseptics and heat modify the physical condition of the soil [König, *et al.* (28), Mann, *et al.* (33), Löhnis (32)].

20. Antiseptics dissolve the soil fats [Greig-Smith (16)], thus aiding the attack upon the soil organic and inorganic matter by microorganisms.

21. Small quantities of antiseptics exert a stimulating effect upon plant growth, as shown by Koch (26, 27), Fred (11, 12) and Oldenbach (40).

22. When heated soil is treated with antiseptics, the favorable influence upon bacterial development and ammonia formation is greatly increased [Greig-Smith (16)].

23. Certain soil organisms, including fungi, actinomycetes and bacteria produce substances toxic to the growth of others [Greig-Smith (16, 17, 18), Nadson and Zolkiewicz (37)]. Antiseptics and heat lead to a neutralization of certain toxic substances present in at least some soils [Greig-Smith (16)].

24. Carbon dioxide formation in soils is also increased as a result of treatment with antiseptics and heat [van Suchtelen (54), Fischer (9)]. At first this is parallel with the increase in bacterial numbers, but the carbon dioxide production soon falls down, while the numbers are still at a high level; this was explained by Fischer as due to the fact that the bacteria pass into a condition of a low respiratory power, but still capable of developing on the plate into colonies. An increase in the amount of oxygen absorbed by the microorganisms of the soil as a result of treatment of soil by heat and volatile antiseptics has been observed by Darbishire and Russell (8).

25. The work of Hiltner and Störmer (21) as well as that of Fischer (9), Moritz and Scherpe (36) suggests that the disinfectant alters the balance of the microbial flora in the soil.

The favorable influence of volatile antiseptics and heat upon the growth of plants and microorganisms has been thus variously explained and the following theories or hypotheses have been suggested.

1. Direct stimulation. Plant roots and microorganisms may be stimulated directly by small quantities of antiseptics [Koch (26), Fred (11)].

2. Indirect stimulation of bacteria. The organic matter in the soil may be modified in such a manner as to make it more available for bacterial action; this may be due either to the removal of the fats [agricere theory of Greig-Smith]; to greater solubility of carbohydrates, nitrogen compounds, or phosphates [Stoklasa (50)]; to the killing of worms, nematodes, protozoa, algae, fungi [Störmer (51)], which are then decomposed by the bacteria; or to all of these combined.

3. Microbiological balance or equilibrium. According to Hiltner and Störmer (21), the results of partial sterilization are due to a change in the bacterial flora.

4. Protozoa theory [Russell and Hutchinson (47, 48)].

5. Toxin theory [Greig-Smith (16)].

6. Destruction of fungi and bacteria which are causative agents of plant diseases [Bolley (2), Jachshevski (24)].

7. Increased nitrogen-fixation [Heinze (19), Hiltner and Störmer (21)]. Koch (27), however, maintains that nitrogen fixation by bacteria is decreased by partial sterilization.

It remains to be seen which of these theories is the correct one and whether the favorable influence of heat and antiseptics both on sick and normal soil can be explained altogether by any one or more of these theories. Even then, according to Miehe [cf. Waget (55)], soil sterilization still presents too many obscurities and uncertainties, so that it cannot be presented as a panacea for soil ills.

#### EXPERIMENTAL

**Methods.** Numbers of bacteria and actinomycetes were determined by the plate method, as described recently (57), using 5 to 10 plates for each determination. The numbers of fungi were determined by the use of a special acid medium (56), using a dilution 0.1 to 0.01 of the final dilution used for the determination of bacteria. The plates for fungi were incubated 48 to 72 hours at 25–28°C. and for bacteria, 6 to 7 days. The protozoa were determined by the dilution method using for the final dilution a 2-per cent hay infusion + 0.5 per cent NaCl and a soil infusion + 0.01 per cent powdered egg-albumen. The cultures were

examined after 5 and 14 days. Only the general groups of ciliates, amoebae and flagellates were determined, without any attempt at further differentiation.

Nitrates were determined by the phenol-disulfonic acid method and ammonia by distillation with  $MgO$ . The production of carbon dioxide in the soil was obtained by placing the proper amount of soil (usually 1 kgm.) in small earthenware pots, bringing the soil to optimum moisture (60 per cent of moisture-holding capacity), then placing the pot under a bell-jar, using an apparatus similar to the one described in detail by Neller (39). The  $CO_2$  was absorbed in standard  $Ba(OH)_2$  solution and titrated back with a standard solution of oxalic acid. A stream of air was passed through the pots at the rate of 3 liters per hour for 14 days, due to the fact that a short period of time may give an incorrect indication of the  $CO_2$  production of the soil. It has been observed by van Suchtelen (54), Potter and Snyder (43) and in our own experiments that, when the soil is placed in the respiration apparatus, the  $CO_2$  production is rapid during the first 2 to 3 days, and then diminishes. It was deemed, therefore, advisable to use a period of 14 days as a unit of time and as an index of  $CO_2$ -producing capacity of the soil. The apparatus was allowed to run all the time, without interruption.

TABLE 1  
*Chemical condition of soils from nitrogen plots*

PLOT NO.	FERTILIZER TREATMENT	CROP YIELD PER ACRE FOR LAST 14 YEARS	NITROGEN CONTENT	CARBON CONTENT	REACTION
		lbs.	per cent	per cent	pH
5A	16 tons cow manure, minerals* . . . . .	60,541	0.1463	1.73	5.5
7A	Nothing . . . . .	15,295	0.0826	0.96	5.0
9A	320 lbs. $NaNO_3$ , minerals . . . . .	50,488	0.0994	1.17	5.8
11A	$(NH_4)_2SO_4$ equivalent to 320 lbs. $NaNO_3$ , minerals . . . . .	38,731	0.1064	1.23	4.6
7B	Lime only . . . . .	27,239	0.0868	1.18	6.3

\* "Minerals" are 320 lbs. potassium chloride and 640 lbs. acid phosphate per acre.

The first experiment to be reported deals with the influence of various concentrations of volatile antiseptics upon bacterial activities of soils of different fertility. In this connection the nitrogen plots of this department, which have received different fertilizer treatments for the last 15 years and where definite differences in fertility have been established have been utilized. A careful record of the crop yields of these plots has been kept and some of these plots have been submitted recently to a careful bacteriological analysis (58). Five plots were used for this purpose. Four 200-gm. portions from the soil of each plot were placed in glass jars. Three of the jars were treated with 0.1, 1.0, and 5.0 per cent of toluene, respectively. The jars were closed tightly with covers and allowed to stand 3 days. At the end of that time, the covers were taken off and the jars plugged with cotton so as to prevent the contamination of the soils with bacteria and fungi floating in the air. The toluene was, therefore, not completely evaporated for some time where the larger amounts of it were used. The moisture content of the soil was kept up by addition of sterile distilled water. Table 1 shows the treatment, crop yield, carbon and nitrogen content and reaction of the plots. The results of the toluene treatment are given in table 2.





11A	0	5.1	9	112	C Fl	5.1	9	112	C Fl
	3	6.8	7	88	C Fl	1.4	20	Tr.	
	12	6.1	7	97	C Fl	1.0	2	0	
	30	2.2	8	118	C Fl	1.4	0	55	
	55	2.6	14	101	C Fl	2.2	0	82	
	NH <sub>4</sub> -N	1.33 mgm.				3.02 mgm.			
	NO <sub>3</sub> -N	1.90 mgm.				0.00 mgm.			
7B	0	6.5	37	9	C	6.5	37	9	C
	3	8.1	25	13	C Fl Am	2.95	32	0.02	Am
	12	8.6	22	7	C Fl	13.6	3	0.05	
	30	7.3	27	22	C Fl	28.2	2	7	
	55	7.08	32	23	C Fl	35.8	0	0.6	
	NH <sub>4</sub> -N	1.12 mgm.				2.24 mgm.			
	NO <sub>3</sub> -N	1.60 mgm.				0.56 mgm.			

\* Counts made just before treatment with toluene.

† Counts made on opening flasks. After this sterile water was added to bring soils to optimum moisture.

‡ B = bacteria; A = actinomycetes; F = fungi; C = ciliates; Fl = flagellates; Am = amoebae.

§ NH<sub>4</sub>-N and NO<sub>3</sub>-N are given as milligrams per 100 gm. of soil after 55 days' incubation.



In the case of the untreated soils, a stimulating effect upon numbers of microorganisms was always obtained as a result of mixing, sieving and bringing up to optimum moisture. This increase was followed, after a few days, by a steady decrease. It is important to note that the percentage of actinomycetes varied usually inversely with the total number of microorganisms developing on the plate, exclusive of fungi, *i.e.*, the percentage of actinomycetes decreased when the total number increased and the actinomycetes ratio began to increase, when the total number began to decrease pointing to the fact that the latter group of organisms is not subject to as rapid changes as the true bacteria developing on the plate. Increases or decreases in the number of actinomycetes take place much more slowly than in the case of bacteria. The numbers of fungi usually tend to increase as a result of incubation in the laboratory, except soil 11A which is already very rich in fungi. All the three major groups of protozoa (ciliates, flagellates, amoebae) were present in the control soils, although the amoebae were not always demonstrated.

In some cases 0.1-per-cent toluene caused a decrease in the number of bacteria followed by an increase or, as in the case of 7A and 7B, it brought about directly a decided increase in the number of bacteria. The protozoa were at first destroyed (except 7B), but later developed again, first the flagellates, then the amoebae and ciliates. It is interesting to note that the greatest stimulus upon the number of bacteria and actinomycetes developing on the plate was brought about in 7B, where the protozoa remained alive and active even after three days treatment with 0.1-per-cent toluene. Although the small amount of toluene was not sufficient to kill the protozoa in this soil, the stimulus exerted upon bacterial development is most pronounced. The fungi were practically destroyed by the 0.1-per-cent toluene and the percentage of actinomycetes was appreciably reduced. The fungi, however, soon began to multiply very rapidly and after 30-50 days greatly exceeded the numbers originally present. After 55 days, as a result of treatment with 0.1-per-cent toluene, sample 5A contained only about 20 per cent more bacteria, more than twice as many actinomycetes and over seven times as many fungi, while the protozoa were as active as at the start; sample 7A contained four times as many bacteria, a smaller percentage of actinomycetes and over five times as many fungi, with the same abundance of protozoa; sample 9A contained three times as many bacteria, a smaller percentage of actinomycetes and seventeen times as many fungi; sample 11A contained a smaller number of bacteria, a higher percentage of actinomycetes and a greater number of fungi; and sample 7B contained four times as many bacteria, much lower percentage of actinomycetes and five and a half times as many fungi.

*A small amount of toluene is thus found to have a destructive effect upon the fungi but a decidedly stimulating effect upon bacteria and temporary depressing effect upon protozoa. By it the soil is made a much better medium for the development of fungi.*

As stated above, the soils were not spread out for the evaporation of the toluene in order to prevent reinoculation with dust fungi; the tight covers were merely replaced by cotton plugs. However, this did not prevent the contamination of the soil and it did prevent the rapid evaporation of toluene in the case of 1.0-per-cent concentration and especially where 5 per cent has been added.

One per cent of toluene produced a much greater depressive effect upon the bacteria and, due to the slow evaporation of the toluene, it took a somewhat longer period of time for bacterial development to take place. The depression was followed in all cases by a rise in numbers reaching in three out of five soils after 55 days a point much above the original number. Here again the soil was made a much better medium for the development of fungi, which reached even higher numbers than in the case of the treatment with 0.1 per cent of toluene. It is interesting to note that in the two soils (5A and 11A), where the numbers of bacteria were after 55 days lower than at start, the numbers of fungi were the highest of all soils at all periods, reaching over one million and one-half million, respectively. While there were at the beginning of the experiment three times as many fungi in 11A as in 5A, 5A contained twice as many fungi as 11A 55 days after treatment with 1 per cent of toluene. Fungi are directly stimulated by the addition of available organic matter. Since 5A has a much greater content of organic matter than 11A, the above results indicate that the toluene treatment brought about a greater availability of the organic matter in the soil (plate 1).

The protozoa were destroyed by 1 per cent of toluene in all soils, but after 55 days they were found again. It is of importance to point out the great abundance of all the three groups of protozoa in 7B, which contains also the highest number of bacteria, but the lowest number of fungi.

In the case of the soils treated with 5 per cent of toluene, the disinfectant was still present after 15-20 days. This lasting effect of the disinfectant resulted in a much greater reduction of the bacteria followed only by a late increase, while the protozoa disappeared completely. Outside of this, one can hardly draw any further conclusions from this treatment, except as to the ammonia content.

The numbers of bacteria, actinomycetes, fungi and protozoa in the soil did not show, as a result of toluene treatment, any correlation with soil fertility, or organic matter content of a soil; as a matter of fact the most fertile soil (5A) showed the least increase in the number of bacteria (and actinomycetes), while the least fertile, but limed, soil showed the greatest increase. This may perhaps be correlated with the inverse development of fungi since 5A showed the greatest development of fungi as a result of toluene treatment and 7B the least. *The formation of ammonia, however, is directly dependent on the organic-matter content of the soil and is not correlated with the increase in the numbers of bacteria, when the differently treated soils are compared.* Soil 5A gave the greatest increase in the amount of ammonia, as a result of toluene

treatment; however the gain over the control is more apparent than real. For 1 and 5 per cent of toluene killed or temporarily put out of action the nitrifying bacteria and the ammonia formed was not nitrified. When we compare the total soluble nitrogen produced, we find 4.94 mgm. for the control, 6.20 mgm. for 0.1 per cent of toluene, 6.93 mgm. for 1 per cent and 6.58 mgm. for the 5 per cent of toluene treatment. The greatest gain is found between the control and 0.1 per cent of toluene treatment; protozoa were found in the latter soil 12 days after treatment, so that surely they had enough time within the 55 days to stop bacterial development. Exactly the same is true, only to a lesser extent of soil 9A. Soils 11A and 7B did not show any appreciable gain of soluble nitrogen in the toluene treated soils over the controls. Soil 7A contained the lowest amount of organic matter and it is only natural to find the lowest amount of ammonia formed, but here again, the toluene treatment did not result in any greater increase in soluble inorganic nitrogen than the control.

The results of the above experiment lead us to conclude that the treatment of an ordinary field soil with toluene will bring about a change in the different ratios between the various groups of soil microorganisms as present in the soil: the total number of bacteria and actinomycetes first decreases as a result of treatment followed by an increase; the larger the amount of toluene applied, the greater will be the decrease in bacterial numbers and the more delayed will be the increase following. The ratio of actinomycetes to the total number of microorganisms developing on the plate decreases with an increase in the latter, but is finally followed by an increase.

Treatment of soil with toluene had a marked influence upon the development of the fungi. These organisms were nearly all destroyed as a result of toluene treatment, but some of them seem to have survived and have developed so rapidly that the numbers soon greatly exceeded those in the original soil. The surviving fungi belonged only to very few species, as could be readily recognized from the plates, perhaps not more than three or four, especially the common soil *Zygorhynchus* and a certain pink *Penicillium*.

Soil treated with toluene is made a better medium for the growth of a few specific fungi. Although the protozoa were at first destroyed, especially the flagellates, they reappeared after some time, depending on the amount of disinfectant applied; in view of the fact that in certain soils (7B) the greatest increase in bacterial development coincides with little injury to protozoa, one might be justified in concluding from these experiments that an increase in the numbers of bacteria and in the decomposition of organic matter in the particular soil is not necessarily correlated with the absence or presence of protozoa.

The above experiment was repeated, with soils from only plots 5A, 7A and 7B. The soil was placed in 2-kgm. portions in large pots and brought to optimum moisture content. One pot of each soil was left as control, two pots were treated with 2 per cent of toluene for 48 hours and two were

heated (by placing in hot water, with thermometer in center of soil) for 1 hour at 65–75°C. After 48 hours the toluened soil was spread out for 4 hours to allow the toluene to evaporate. The soil was then returned to the pots and brought back to weight with sterile distilled water. One of the two treated pots was left without further treatment and to the other one 5 per cent of fresh soil was added and well mixed in. The pots were then kept covered in the incubator at 25–28°C. At frequent intervals, samples were taken for the determination of numbers of bacteria and actinomycetes, fungi, ammonia- and nitrate-nitrogen. The pots were kept at the same moisture content. Results are given in table 3.

Here again we find that the control soils (5A and 7B), when brought under optimum moisture and temperature conditions, show at first a slight increase in the number of microorganisms, followed by a constant decrease. Soil 7A shows more or less uniform numbers for the first month. The discrepancy in the behavior of 7A in this experiment from the previous one may be due to the fact that this time the soil was sampled in April when the moisture was at an optimum, while the previous experiment was carried on in the fall. The ammonia in the control soil is present always only in small amounts while, on continued incubation, it even tends to diminish because of its transformation into nitrates. The latter continuously accumulate in the incubated untreated soil. The total soluble nitrogen constantly increases in the manured soil (5A) which is rich in organic matter, while it remains on a level in the two unmanured plots. At the beginning all the three plots contained about 2 mgm. of soluble nitrogen in 100 gm. of soil; at the end of 31 days' incubation, 5A contained about twice as much soluble nitrogen, or 3.68 mgm., while 7A and 7B contained only 1.84 and 2.06 mgm. respectively, or just about the same as when freshly sampled.

Toluene treatment exerted a characteristic influence upon the numbers of microorganisms in the soil and upon ammonia accumulation, similar to that observed by other investigators and in our first experiment. The total numbers were depressed as a result of the treatment, but soon began to increase greatly exceeding the untreated soil. The same is true, but to a much more striking extent of the fungi. Toluene almost completely depressed this group of organisms in the soil but they soon began to multiply again, especially so in the reinoculated soil. The three soils behave differently after toluene treatment. The slightly acid soil 5A, rich in organic matter, allowed only a fair increase in the number of bacteria (including the actinomycetes) so that in 30 days this soil contained over twice as many bacteria as the untreated control soil. The numbers of fungi which were diminished from 34,000 to 65 per gram or practically completely repressed as a result of toluene treatment, have increased in the uninoculated soil to 132,000 and in reinoculated soil to 190,000 per gram; *i.e.*, to four and nearly six times the number of those of the control soil. In 7B, the neutral, limed soil, rather poor in organic matter, the numbers of bacteria, originally less than in 5A, were repressed to a



PLOT NUMBER	LENGTH OF INCUBA- TION PERIOD days	HEATED (65-75° C. FOR 1 HOUR)									
		Uninoculated					Reinoculated with 5 per cent of fresh soil				
		B + A	A	F	N in 100 gm. soil		B + A	A	F	N in 100 gm. soil	
		thousands	per cent	thousands	NH <sub>4</sub> -N mgm.	NO <sub>3</sub> -N mgm.	thousands	per cent	thousands	NH <sub>4</sub> -N mgm.	NO <sub>3</sub> -N mgm.
5A	0†	1,600	12		2.00	0.26	1,100	4	0.13	1.68	0.29
	7	10,600	17	8	3.74	0.25	42,600	22.5	83	3.17	0.35
	15	12,000	15	43	4.61	0.30	45,000	24	160	3.74	0.76
	31	11,000	17	68	5.18	0.20	58,000	22	170	3.17	2.40
	43	24,000	10	56	5.04	0.21	51,000	39	172	1.58	4.80
	60	33,000	9	57	5.18	0.04	43,500	42	167	0.72	5.30
7B	105	18,500	12	146	1.80	5.90	23,600	42	140	0.50	6.50
	0	1,700	13	0.065	1.68	0.06	900	3		1.68	0.06
	7	27,000	8	0.6	2.30	0.08	46,400	4	7.3	3.17	0.12
	15	42,000	5	1	2.30	0.13	48,000	2	38	3.17	0.17
	31	33,000	10	0.7	1.73	1.50	28,500	6	32	2.06	1.70
	43	26,000	10	3	1.44	1.70	33,500	7	46	0.72	2.10
	60	35,500	6	0.7	0.87	1.40	24,000	4	8.3	0.29	1.60
	105	26,000		0.7	0.20	3.20	23,400	4	9.3	0.10	3.20

\* B + A = bacteria and actinomycetes; A = actinomycetes, per cent of colonies developing on plate; F = fungi; NH<sub>4</sub>-N = ammonia nitrogen; NO<sub>3</sub>-N = nitrate nitrogen.

† Incubation was started soon after toluene was evaporated or heating of soil was completed.



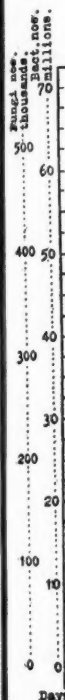
greater extent by the toluene; this was followed by an increase so that in 15 days there were 35,000,000 and 70,000,000 in the uninoculated and reinoculated soils respectively; *i.e.*, three and a half and seven times as much as those of the control. The fungi, however, in this very soil, multiplied only very slowly even when reintroduced. The microbial flora in 7A behaved in a similar way as in 5A, never reaching such large numbers as in the latter undoubtedly because of the lower content of organic matter.

It is interesting to note here that 5A was found to contain after 15 days' incubation both amoebae and flagellates in the uninoculated and reinoculated soils, while 7B contained flagellates in the reinoculated soil and no protozoa in the uninoculated soil. Soil 7A was free from protozoa. In 43 days, there was an abundance of protozoa, chiefly small amoebae and flagellates in all the soils. As a matter of fact, the toluene-treated and reinoculated soils contained larger numbers of amoebae than the control soils. The uninoculated soils contained fewer protozoa. There was no correlation, however, between the numbers of bacteria and development of protozoa.

There was a slight increase in ammonia as a result of toluene treatment, especially in soil 5A. The ammonia continuously increased in soil 5A, both in the uninoculated and reinoculated soils, while the nitrifying bacteria became active in both cases only after 60 days. The increase in ammonia was only slight in soil 7A, while it actually decreased in the reinoculated soil. There was a definite increase in ammonia content in soil 7B, a part of which was converted into nitrates in the reinoculated soil, in which the nitrifying bacteria became more active than in the unlimed soils, once reintroduced.

When we compare the curves for bacteria, fungi and ammonia (fig. 1, 2, 3) for soils 5A, 7A, and 7B, we find that the ammonia accumulation does not go hand in hand with the increase in bacteria: but that there is much less ammonia formed in soil 7B with a much greater bacterial flora than in soil 5A. We could explain the ammonia accumulation by the fungus curve, but even this would not be the whole explanation.

Soil 5A is rich in organic matter and acid in reaction, therefore it favors fungus development; soil 7A is even more acid in reaction but poor in organic matter, and we would, therefore, expect that any fungus development taking place in this soil would be at the expense of the soil nutrients; soil 7B is nearly neutral in reaction and is poor in organic matter, we would then expect a limited fungus development, independent, of course, of the development of protozoa and bacteria. The latter, however, are markedly influenced by the development of fungi. A high fungus flora in a soil rich in organic matter (5A) seems to have some relation to the rather limited increase in bacteria, in comparison with a soil (7B) not favoring the development of fungi. The accumulation of ammonia depends upon the abundance of organic matter in the soil, the amount of ammonia used up by the soil organisms and that transformed into nitrates. Soil 5A, rich in organic matter allows a large development of bacteria and fungi and a high accumulation of soluble nitrogen.





Soil 7A, acid in reaction and low in organic matter, allows a limited development of bacteria, a high development of fungi and, as a result of these two phenomena, a low accumulation of soluble nitrogen. Soil 7B, neutral in reaction but low in organic matter, allows a high development of bacteria, low development of fungi, and, as a result of these two phenomena, a comparatively high accumulation of soluble nitrogen.

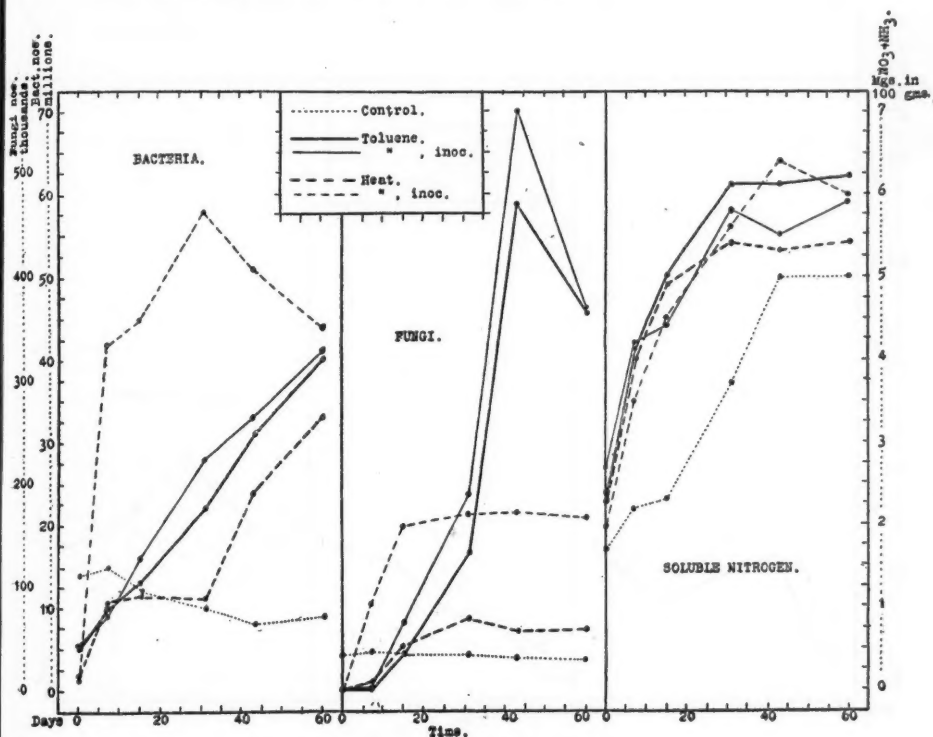


FIG. 1. INFLUENCE OF TOLUENE AND HEAT UPON NUMBERS OF BACTERIA, FUNGI AND ACCUMULATION OF SOLUBLE NITROGEN IN AN ACID SOIL RICH IN ORGANIC MATTER (5A)

Heating the soil produced about the same effect as treatment with toluene. Bacteria increased at first in 5A only to a limited extent in the uninoculated soil, but very markedly when the soil was reinoculated. Fungi increased in both cases rapidly, more so in the reinoculated soil. Bacteria increased rapidly in soil 7B in both uninoculated and reinoculated soils, while the fungi multiplied rapidly only when reinoculated. A great deal of ammonia was formed in soil 5A, a part of which was transformed into nitrates in the reinoculated

soil. Some ammonia was formed also in soil 7B, less in the uninoculated than the reinoculated soil, possibly due to the more abundant development of fungi in the latter. The nitrifying bacteria became active in the heated soil more rapidly than in the toluene-treated soil, especially when reinoculated. A detailed discussion of the metabolism of bacteria, fungi and actinomycetes as bearing upon these results will be published later.

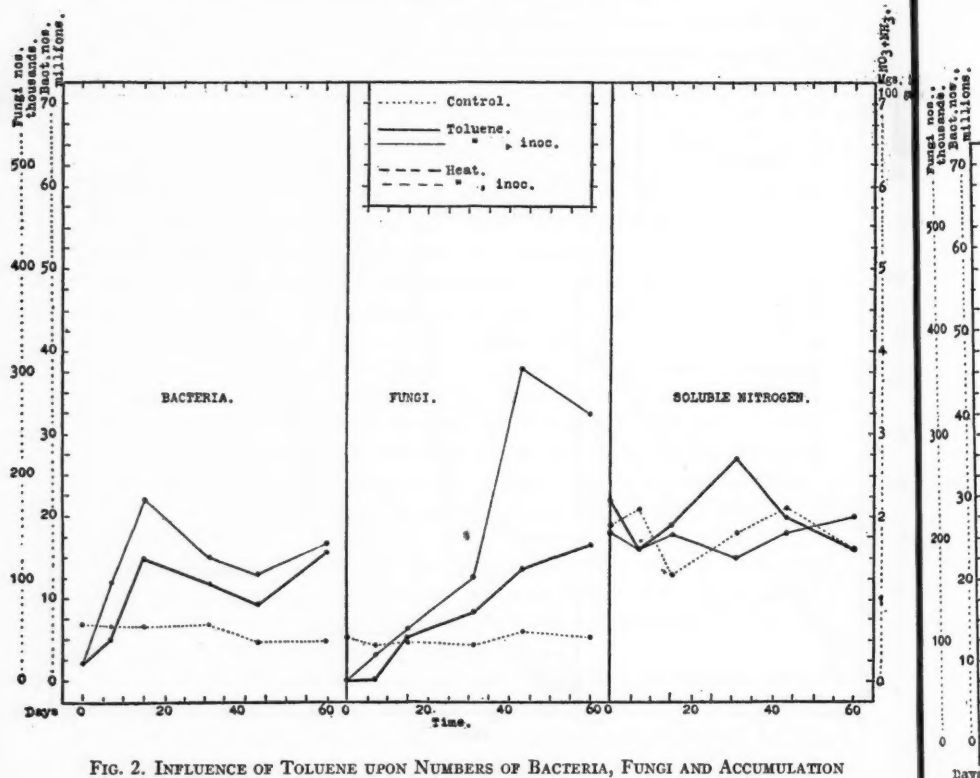


FIG. 2. INFLUENCE OF TOLUENE UPON NUMBERS OF BACTERIA, FUNGI AND ACCUMULATION OF SOLUBLE NITROGEN IN AN UNLIMED ACID SOIL POOR IN ORGANIC MATTER (7A)

#### SUMMARY

1. The results of a study on the influence of toluene and heat upon the microbiological population of variously treated soils are reported.
2. Toluene and heat influence markedly not only the development of bacteria and protozoa in the soil but also that of the actinomycetes and especially the fungi.
3. The results on the influence of toluene and heat upon bacteria and protozoa agree with those of previous investigators.

4. The fungi are repressed very markedly by toluene and heat, but soon begin to develop, especially when reintroduced, and reach numbers greatly in excess of the untreated soil. The fungi developing in the partially sterilized soils are limited to only a few species.

5. Ammonia accumulates in the partially sterilized soils in excess over the untreated soil, especially in soils rich in organic matter, but in poorer soils the rapid development of fungi may lead to a partial utilization of the ammonia.

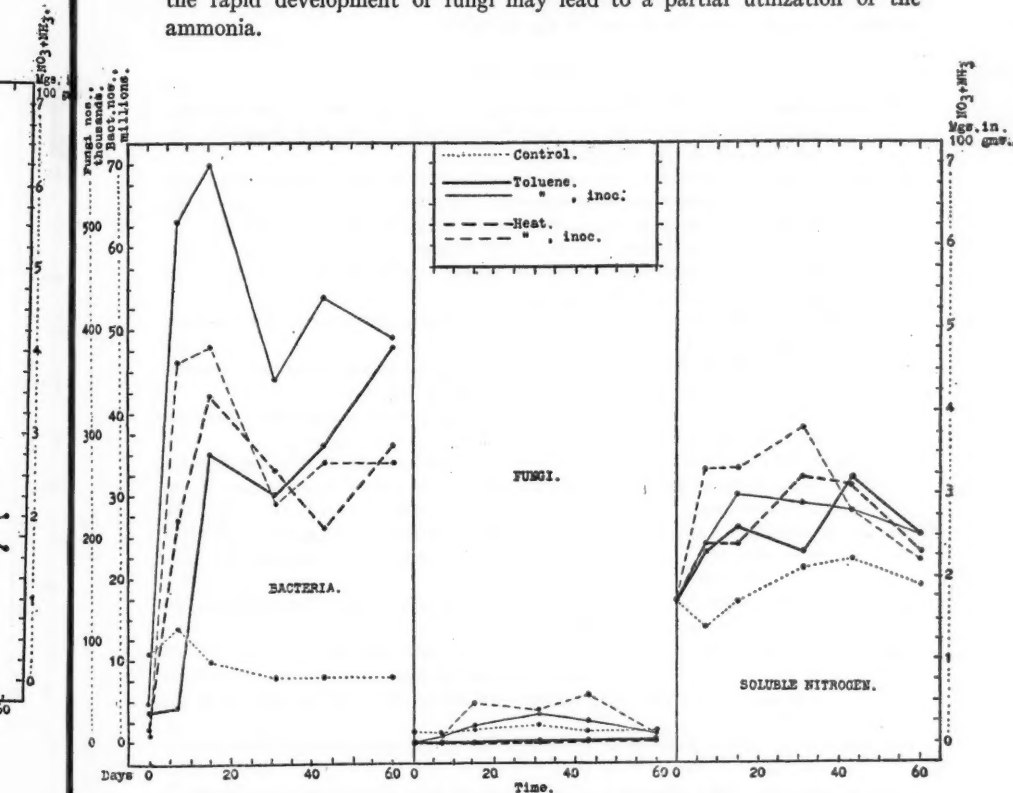


FIG. 3. INFLUENCE OF TOLUENE AND HEAT UPON NUMBERS OF BACTERIA, FUNGI AND ACCUMULATION OF SOLUBLE NITROGEN IN A LIMED, BUT SLIGHTLY ACID SOIL POOR IN ORGANIC MATTER (7B)

6. There is no correlation between the increase in bacterial numbers and accumulation of ammonia, when we compare soils containing different amounts of organic matter.

7. It is possible that the protozoa become a limiting factor to bacterial development in certain abnormal soils, such as sewage-treated soils or cer-

tain greenhouse soils, where the soil is practically saturated with moisture and in the presence of a great abundance of organic matter. However, in the case of normal soils, we should consider the part played by fungi and actinomycetes, in addition to bacteria, in the decomposition of organic matter, the influence of the abundant development of those organisms upon bacterial numbers, the influence of kind and amount of organic matter upon the formation of ammonia and multiplication of bacteria, before we can attempt to explain the limitation of the bacteria.

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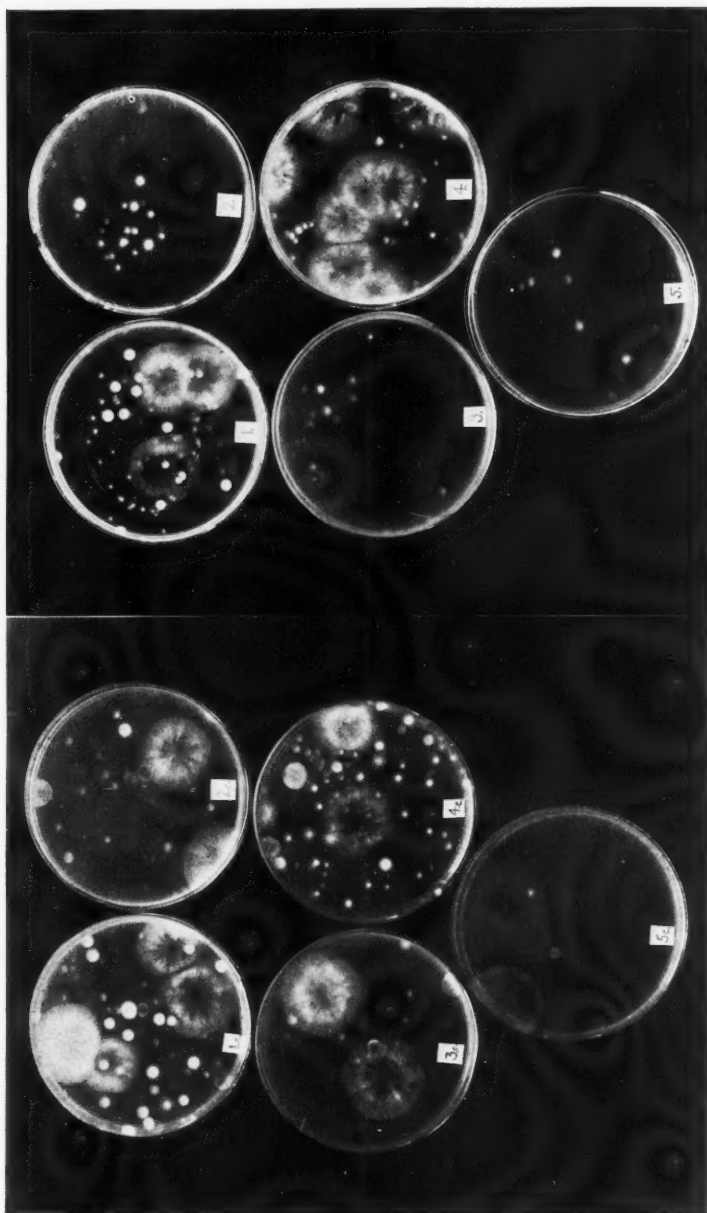
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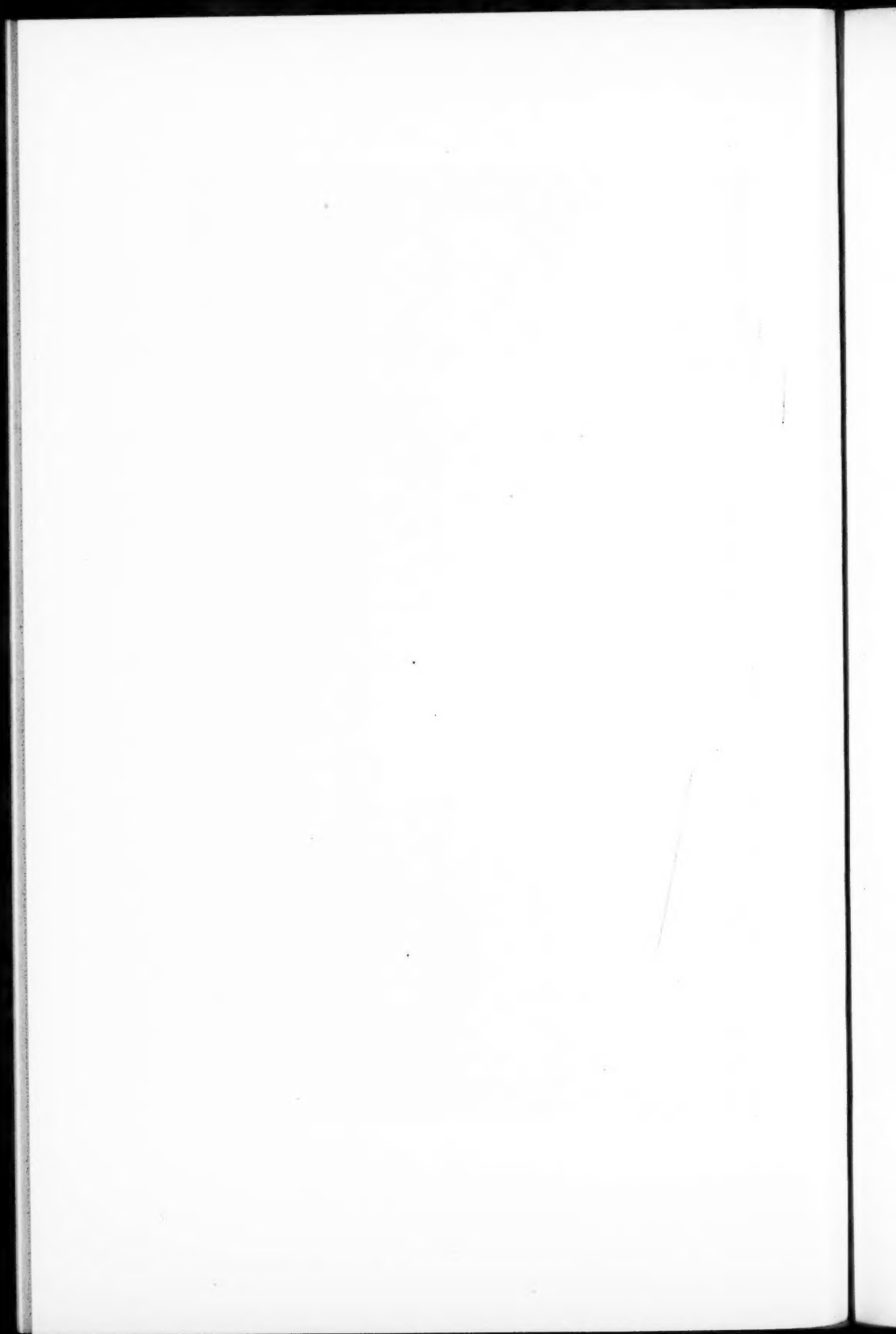
## PLATE 1

## INFLUENCE OF 1 PER CENT OF TOLUENE UPON THE FUNGOUS FLORA OF THE SOIL

The control jars are marked "c." The dilution for the toluene-treated pots was 10,000; for the controls, 1000.







## INFLUENCE OF CALCIC AND MAGNESIC TREATMENTS UPON SULFATE LEACHINGS FROM SOIL ALONE AND WITH ADDI- TIONS OF FERROUS SULFATE, PYRITE, AND SULFUR

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In a preliminary report (2) and a subsequent detailed contribution (6) upon the leaching of soil sulfates over an 8-year period, the influence of the oxides and carbonates of calcium and magnesium were shown to be divergent. That study had to do only with native materials, impurities of treatment, and rainfall, as sources of sulfates. Furthermore, the smallest additions were at the rate of 8 tons of CaO equivalence. It seemed desirable to ascertain whether added sulfureous materials are also subject to the divergent activities of calcium and magnesium. It was thought well to determine also the relative activities of economic applications of oxides and native carbonates upon soil without sulfur additions. Accordingly, in August, 1917, 3 years after the installation of the 46 lime-magnesia tanks, 22 additional lysimeters were placed. The sulfate leaching data secured from these 22 tanks during the first 5-year period are given in this contribution.

### METHODS AND MATERIALS

While the annual periods for collection of leachings were from August to August, the annual rainfall collections were made quarterly. The 1917-1922 sulfate precipitations previously reported (5) are used in determining the amount of sulfate sulfur brought to the soil.

The tanks were filled August 2, 1917, with a loam soil designated as "Cherokee." It was acid to litmus and showed an electrometrically determined pH value of 6.27; ratio 1-10; one hour's contact. Sodium peroxide oxidation and disintegration showed an  $\text{SO}_3$  content of 0.0815 per cent. One-foot tanks only were used, since the earlier work (2,6) had definitely established the function of the subsoil in changing the concentration of sulfate leachings under different conditions of treatment. The depth of soil in good tilth was about 8 inches. Each tank embraced an area of 1/20,000 acre and received 118.7 pounds of moist soil, equivalent to 100 pounds, moisture-free basis. The moist soil was screened, mixed and protected overnight during the determination of moisture upon a large sample. The alkali-earth treatments were all based upon equivalence of the designated number of pounds, or tons, of CaO per acre 2,000,000 pounds of moisture-free soil.

Three sulfur materials were used, ferrous sulfate, pyrite, and pulverized sulfur. These three materials carried 18.15 per cent, 51.95 per cent, and 99.89 per cent of sulfur, respectively. Each was applied in an amount equivalent to 1000 pounds of S per acre 2,000,000 pounds of soil. The combination afforded an opportunity for study of the outgo of sulfates from

a readily soluble application carrying the sulfate radical, as compared with pyrite, which upon oxidation yields the same soluble sulfate, plus  $\text{SO}_2$ , and the elementary form, without introducing any alkali addition. At the time of the inauguration of this experiment, insofar as known to us, no lysimeter investigation had been directed toward the availability of pyrite as a source of sulfur in soils. This material was included, therefore, as representing a cheap possible source of sulfur, in comparison with "flowers." The treatments are shown in the following outline:

*Outline of calcium, magnesium, and sulfur additions*

<i>Tank No.</i>	<i>Treatment</i>
50	Untreated soil
51	Ground limestone, 100-mesh, equivalent to 2,000 pounds CaO per acre
52	Ground dolomite, 100-mesh, equivalent to 2,000 pounds CaO per acre
53	Burnt lime, 2,000 pounds per acre
54	Burnt lime, 3,750 pounds per acre
55	Burnt magnesia, equivalent to 2,000 pounds CaO per acre
56	Burnt magnesia, equivalent to 3,750 pounds CaO per acre
57	$\text{FeSO}_4$ , equivalent to 1,000 pounds of S per acre
58	$\text{FeSO}_4$ , as in 57, and 3,750 pounds of CaO per acre
59	$\text{FeSO}_4$ , as in 57, and MgO equivalent to 3,750 pounds of CaO per acre
60	$\text{FeSO}_4$ , as in 57, and 32 tons of CaO per acre
61	$\text{FeSO}_4$ , as in 57, and MgO equivalent to 32 tons of CaO per acre
62	$\text{FeS}_2$ (pyrite), equivalent to 1,000 pounds of S per acre
63	$\text{FeS}_2$ (pyrite), as in 62, and 3,750 pounds of CaO per acre
64	$\text{FeS}_2$ (pyrite), as in 62, and MgO equivalent to 3,750 pounds of CaO per acre
65	$\text{FeS}_2$ (pyrite), as in 62, and 32 tons of CaO per acre
66	$\text{FeS}_2$ (pyrite), as in 62, and MgO equivalent to 32 tons of CaO per acre
67	Sulfur, 2,000 pounds S per acre
68	Sulfur, as in 67, and 3,750 pounds of CaO per acre
69	Sulfur, as in 67, and MgO equivalent to 3,750 pounds of CaO per acre
70	Sulfur, as in 67, and 32 tons of CaO per acre
71	Sulfur, as in 67, and MgO equivalent to 32 tons CaO per acre

It was planned to determine activation in sulfate generation through comparison between chemically equivalent amounts of limestone and dolomite at the rate ordinarily used in practice and a further joint comparison between these two materials and both burnt lime and magnesia upon the native sulfur materials in the soil. It was also intended to compare the effect of 1-ton and 32-ton admixtures of both CaO and MgO, where sulfur additions were made. The latter amount was taken as the maximum, in lieu of 100 tons, since the earlier work showed that the two heavy rates manifested the same tendencies. The limestone used was sulfur-free. The dolomite added sulfur at the rate of 35 pounds per 2,000,000 pounds of soil. The 2000-pound, 3750-pound, and 32-ton CaO additions showed impurities equivalent to 0.8 pound, 1.7 pounds, and 25.6 pounds, respectively. In the three corresponding additions of MgO the sulfur impurity amounted to 2.6 pounds, 4.9 pounds, and 83.2 pounds. Only the 25.6-pound impurity of the heavy CaO treatment and that of 83.2 pounds in the heavy MgO addition need be considered.

In order to have an actual supplement of 2000 pounds of CaO, it was necessary to take into account the immediate acidity of the  $\text{FeSO}_4$  and also the potential acidity of the un-

oxidized sulfur materials. The 32-ton CaO and MgO treatments were sufficiently excessive to minimize the difference between immediate and potential acidity. The lime necessary to care for such acidity was therefore added as supplementary to the 2000-pound addition, making 3750 pounds, or its equivalent, the supplementary minimum for sulfur carriers.

TABLE 1

*First annual outgo of sulfates from Cherokee sandy loam—with and without sulfur additions—as influenced by calcic and magnesian materials\**

TANK NUMBER	TREATMENTS PER 2,000,000 LBS. OF SOIL			TOTAL LEACHINGS AVERAGE OF BaSO <sub>4</sub> PER LITER FROM PERIODIC DETERMINATIONS		S LOST IN LEACHINGS FROM AN ACRE SURFACE EXPOSURE, 2,000,000 LBS. OF SOIL					
	Calcic-magnesic		Equivalence of 1000 lbs. of S			Sept. 29, 1917	Jan. 29, 1918	Feb. 19, 1918	April 22, 1918	Aug. 3, 1918	Total 1917-1918
	Material	CaO equivalent lbs. or tons									
				liters	gm.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
50	None	None	None	85.7	0.0334	1.3	2.4	5.2	6.0	3.1	18.0
51	Limestone	2000 lbs.	None	85.1	0.1128	9.0	7.9	14.2	21.8	4.9	57.8
52	Dolomite	2000 lbs.	None	85.3	0.1230	8.5	9.9	15.0	23.3	5.5	62.2
53	CaO	2000 lbs.	None	84.6	0.1223	8.3	9.3	15.9	22.9	5.8	62.2
54	CaO	3750 lbs.	None	76.2	0.1812	10.5	13.6	23.2	26.2	7.1	80.6
55	MgO	2000 lbs.	None	84.3	0.1398	9.7	10.6	14.5	21.2	6.7	62.7
56	MgO	3750 lbs.	None	79.6	0.1936	13.7	11.0	32.6	29.3	7.0	93.6
57	None	None	FeSO <sub>4</sub>	75.2	1.3037	135.6	55.1	148.3	151.5	43.8	534.3
58	CaO	3750 lbs.	FeSO <sub>4</sub>	73.8	1.8867	138.3	82.7	236.8	300.5	71.1	829.4
59	MgO	3750 lbs.	FeSO <sub>4</sub>	71.5	2.7464	258.4	160.4	312.6	161.8	30.7	923.9
60	CaO	32 tons	FeSO <sub>4</sub>	77.7	0.1747	36.1	20.1	6.3	5.7	14.0	82.2
61	MgO	32 tons	FeSO <sub>4</sub>	54.9	3.3142	77.1	262.7	187.0	409.5	70.8	1007.1
62	None	None	Pyrite	83.7	0.3661	14.7	23.7	53.6	80.2	25.1	197.3
63	CaO	3750 lbs.	Pyrite	77.3	0.4025	25.6	19.3	40.9	69.0	34.5	189.3
64	MgO	3750 lbs.	Pyrite	79.7	0.3989	27.3	29.9	25.3	76.6	24.5	183.6
65	CaO	32 tons	Pyrite	84.4	0.0611	9.4	8.3	2.2	4.2	7.4	31.5
66	MgO	32 tons	Pyrite	62.1	0.4258	11.5	27.6	39.1	71.0	16.3	165.5
67	None	None	Sulfur	81.6	1.0504	55.4	69.8	149.4	188.9	43.3	506.8
68	CaO	3750 lbs.	Sulfur	73.7	1.6422	96.9	66.2	195.2	287.4	87.8	733.5
69	MgO	3750 lbs.	Sulfur	79.4	1.7062	101.3	204.6	160.2	284.0	66.5	816.6
70	CaO	32 tons	Sulfur	88.5	0.1740	17.8	19.9	8.0	8.6	34.3	88.6
71	MgO	32 tons	Sulfur	55.8	3.0761	28.4	76.9	0.0†	259.5	61.0	425.8

\* Rainfall—August to August 37.69 inches—S precipitated, 43.6 pounds.

† No leaching

It was difficult to anticipate the period required for oxidation of pyrite and sulfur; hence the possibility of a considerable difference between the amount of residual lime or magnesia in the tanks which received the 3750-pound basic treatment and the equivalence of free sulfuric acid and those which received the same basic supplement to the unoxidized sulfur

materials of potential acidity. Accordingly, such an experimental discrepancy was provided for by no-sulfur-addition controls which received lime and magnesia at the two rates of 2000 pounds and 3750 pounds.

In the earlier work where no sulfur was added, it was difficult to determine definitely whether the initial minimum and subsequent increase of sulfate leachings from heavy burnt lime additions were due to initial bacterial inhibition and subsequent recovery, or to depressed solubility of calcium sulfate as long as the soil water was impregnated with  $\text{Ca}(\text{OH})_2$ , or to physical and chemical changes in the soil as consequent to the conversion of the excess of hydrate to carbonate. It was hoped that the inclusion of ferrous sulfate would clarify this phase of the problem.

In the earlier investigation the effect of calcic and magnesian materials upon sulfur outgo was incidental to the main problem; hence, sulfate determinations were made only upon the annual composite of leachings. In the present study, however, the sulfate outgo was of primary interest, so that determinations of sulfates were made upon every periodic collection of leachings throughout the 5-year period. For the sake of brevity, the analytical results of tables 1-6 are expressed only as annual average  $\text{BaSO}_4$ -per-liter determinations and as pounds per acre for the separate leachings.

#### SULFATE LEACHINGS DURING THE FIRST YEAR

*No-sulfur additions.* Limestone and dolomite both caused a very decided increase in the leaching of sulfates in the several collections throughout the year. At the 1-ton rate,  $\text{CaO}$  and  $\text{MgO}$  gave practically identical yields; but at the 3750-pound rate, both oxides caused a heavier yield of soluble sulfates with the  $\text{MgO}$  giving greater response. These relationships held not only for annual outgo, but also for each of the 5 collections of drainage water. The 18-pound loss from the control was less than the 43.6-pound sulfur precipitation for the year, but the outgo from each of the 6 treatments was in excess of rainfall sulfur. It is therefore apparent that the treatments have either induced the passage of all, or much of the rain water sulfur, or they have generated additional sulfates from the sulfur materials in the soil, unless it be that they have forced out sulfates previously resistant to leaching.

*$\text{FeSO}_4$  additions.* The five collections from the sulfate control gave a total recovery slightly more than one-half of the  $\text{FeSO}_4$  addition, as a result of the subnormal annual precipitation of 37.69 inches. Both supplementary treatments of  $\text{CaO}$  and  $\text{MgO}$  caused leachings very much heavier than those from the control, not only as totals, but also for all separate leachings, save one, the fifth and smallest. Eighty-one per cent of the addition is accounted for by the light  $\text{CaO}$  treatment and 91 per cent by the corresponding  $\text{MgO}$  treatment, after deducting the recovery obtained from the no-treatment control. An entirely different parallel appears, however, in the case of the 32-ton additions. Though added in soluble form at the beginning of the experiment, the sulfate addition did not come out in the leachings. The  $\text{CaO}$  so depressed the outgo of the incorporated soluble sulfate that the total for the year was only 10 per cent of that which came from the 1-ton plus supplement. This finding and similar results from the same lime supplement to the pyrite and sulfur treatments will be considered jointly in discussing chemical and

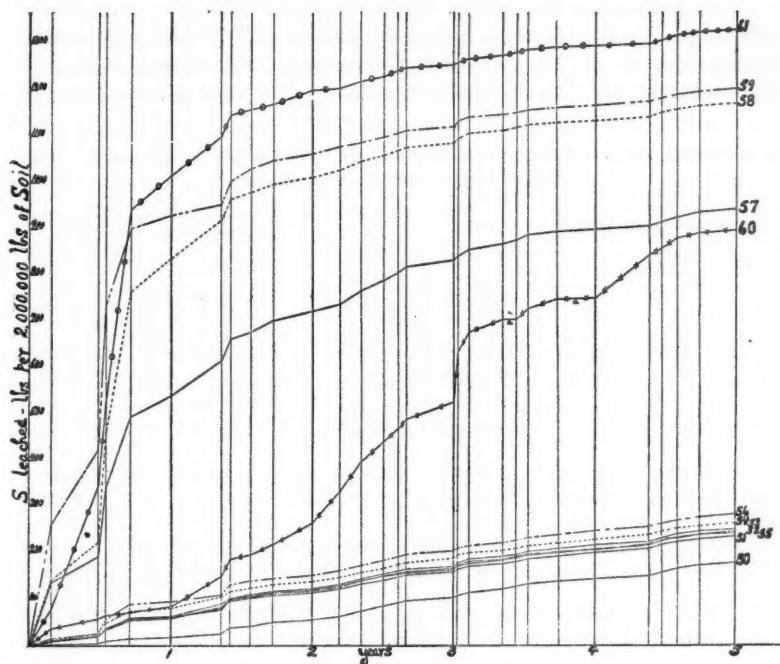


FIG. 1. INFLUENCE OF CaO, MgO, LIMESTONE AND DOLOMITE UPON LEACHING OF SULFATES DERIVED FROM SOIL AND RAINFALL AND RECOVERY OF ADDED  $\text{FeSO}_4$ , WITH AND WITHOUT CaO AND MgO, AS DETERMINED FROM PERIODIC LEACHINGS OVER A 5-YEAR INTERVAL

No sulfur		Ferrous sulfate	
Tank 50, no supplement.....	————	Tank 57, no supplement.....	————
Tank 51, limestone, 2,000-lb. CaO equivalent.....	————	Tank 58, CaO, 2,000 lbs. (+ 1750) .....	-----
Tank 52, dolomite, 2,000-lb. CaO equivalent.....	————	Tank 59, MgO, 2,000-lb. CaO equivalent (+ 1750) .....	-----
Tank 53, CaO, 2,000 lbs.....	————	Tank 60, CaO, 32 tons.....	x-x-x-x
Tank 54, CaO, 3,750 lbs.....	-----	Tank 61, MgO, 32-ton CaO equivalent.....	o-o-o-o
Tank 55, MgO, 2,000-lb. CaO equivalent.....	————		
Tank 56, MgO, 3,750-lb. CaO equivalent.....	-----		

biochemical causes and in connection with previous work. On the other hand, the heavy MgO treatment was responsible for almost a full recovery, 98.91 per cent, of the added sulfate after deduction of the outgo from the no-treatment control.

*Pyrite conditions.* The control of this treatment gave an outgo which indicates an oxidation of 19 per cent of the addition. The reaction responsible for the oxidation of  $\text{FeS}_2$  into  $\text{FeSO}_4$  also generates  $\text{SO}_2$ , equal quantities of S being accounted for by the two end-products. It is more probable that the

TABLE 2

*Second annual outgo of sulfates from Cherokee sandy loam—with and without sulfur additions—as influenced by calcic and magnesian materials\**

TANK NUMBER	TREATMENTS PER 2,000,000 LBS. OF SOIL			TOTAL LEACHINGS	AVERAGE OF BaSO <sub>4</sub> PER LITER FROM PERIODIC DETERMINATIONS	S LOST IN LEACHINGS FROM AN ACRE SURFACE EXPOSURE, 2,000,000 LBS. OF SOIL					
	Calcic-magnesic		Equivalence of 1000 lbs. of S			Dec. 11, 1918	Jan. 3, 1919	Feb. 24, 1919	April 22, 1919	Aug. 4, 1919	Total 1918-1919
	Material	CaO equivalence lbs. or tons									
50	None	None	None	131.6	0.0444	8.4	11.7	1.8	9.7	5.6	37.2
51	Limestone	2000 lbs.	None	130.5	0.0677	24.9	12.4	5.3	5.9	6.6	55.1
52	Dolomite	2000 lbs.	None	130.9	0.0670	26.8	12.4	4.9	7.1	4.8	56.0
53	CaO	2000 lbs.	None	131.1	0.0706	18.6	19.8	6.7	7.6	5.8	58.5
54	CaO	3750 lbs.	None	122.3	0.0728	24.5	12.7	4.7	6.9	7.4	56.2
55	MgO	2000 lbs.	None	124.4	0.0677	15.0	19.9	4.4	7.1	7.0	53.4
56	MgO	3750 lbs.	None	124.9	0.0720	14.7	21.9	7.6	5.8	6.6	56.6
57	None	None	FeSO <sub>4</sub>	124.3	0.2249	67.9	54.1	14.7	25.7	18.5	180.9
58	CaO	3750 lbs.	FeSO <sub>4</sub>	121.7	0.2206	83.3	43.2	14.8	18.2	13.6	173.1
59	MgO	3750 lbs.	FeSO <sub>4</sub>	118.0	0.1783	22.3	50.7	24.3	18.1	13.8	129.2
60	CaO	32 tons	FeSO <sub>4</sub>	109.4	0.2839	66.7	35.9	8.7	22.0	45.1	178.4
61	MgO	32 tons	FeSO <sub>4</sub>	102.2	0.2133	84.8	46.6	10.6	10.9	31.0	183.9
62	None	None	Pyrite	124.4	0.3407	139.2	64.4	5.6	36.7	32.9	278.8
63	CaO	3750 lbs.	Pyrite	126.6	0.3115	151.1	42.4	4.6	19.1	45.9	263.1
64	MgO	3750 lbs.	Pyrite	128.2	0.3123	149.1	36.9	19.7	11.9	37.0	254.6
65	CaO	32 tons	Pyrite	132.0	0.0793	23.4	12.8	2.0	10.2	18.7	67.1
66	MgO	32 tons	Pyrite	107.4	0.2315	43.2	33.5	14.3	9.5	6.7	107.2
67	None	None	Sulfur	130.7	0.2504	80.6	67.7	19.8	26.0	16.9	211.0
68	CaO	3750 lbs.	Sulfur	122.7	0.2977	121.9	64.6	15.6	19.1	14.5	235.7
69	MgO	3750 lbs.	Sulfur	123.5	0.2708	137.8	37.9	13.5	15.0	14.4	218.6
70	CaO	32 tons	Sulfur	122.9	0.2570	82.7	28.5	2.8	21.6	69.6	205.2
71	MgO	32 tons	Sulfur	102.6	0.8640	395.4	154.2	26.1	18.9	12.9	607.5

\* Rainfall August to August 51.41 inches—S precipitated, 45.0 pounds.

moisture conditions of the soil and supplies of basic silicates, together with oxidative processes, are responsible for the conservation of the generated  $\text{SO}_2$  as dissolved sulfates rather than permitting its loss in the gaseous phase. The



inclusion of CaO and MgO at the 2000-pound-plus rate showed but little effect, 18 per cent oxidation having been indicated by the leachings from these two treatments. The additions of the two bases still further decrease the probability of loss of the  $\text{SO}_2$  generated in the process of oxidation of  $\text{FeS}_2$ . The heavy CaO again showed distinct depressive influence upon sulfate outgo. The total recovery of 31.5 pounds was but little more than the loss from the no-sulfur control and less than the sulfur content of the annual precipitation. Indication of depressed oxidation is evidenced by the retardation of sulfate leachings from the heavy MgO addition, in comparison with the pyrite control and light treatments, and this indication finds substantiation in the still more striking results of the four succeeding years.

*Sulfur additions.* The sulfate sulfur outgo from the sulfur control was practically one-half of the amount introduced through treatment. Acceleration in outgo, as indicative of increased sulfonation, was caused by the light treatments of both CaO and MgO, the latter having proved the more active. Once more the heavy burnt lime showed the smallest sulfate yield, although its leachings carried more than those from the same treatment used with either sulfate or pyrite and also five-fold more than the leachings from the no treatment control. The heavy MgO addition gave a large recovery, though one considerably less than that of the sulfur control. It probably should be explained that the last leaching of the year from this treatment was very much smaller than the leachings from the other four tanks of the same group. There were no collections between August 4, the end of the first year, and December 11, when the first collections of the second year were obtained. This first collection of the second year was heavily impregnated with sulfates, carrying the per acre equivalent of 395.4 pounds of sulfate sulfur. When this amount is added to the total of the first year and the control is handled in the same way, an accelerative influence is shown for the heavy MgO addition.

#### SULFATE LEACHINGS DURING THE SECOND YEAR

*No-sulfur additions.* The loss from the control through leachings caused by the 51-inch rainfall was greater than that caused by the smaller and subnormal precipitation of the first year. The control outgo was still less than the content of rainfall and considerably less than the rather closely agreeing losses from the four 2000-pound and two 3750-pound calcic and magnesian additions.

*$\text{FeSO}_4$  additions.* The sulfate recovery from the  $\text{FeSO}_4$  control was nearly the same as that from the light CaO treatment in spite of the large excess released by the latter during the first year. But the still greater excess initially shown by the light MgO was reflected in the decrease below that of the control. This tendency toward decrease was not manifested, however, by the heavy MgO addition. This may be due in part to activated oxidation of native materials and impurities, or accelerated movement of rainfall. The sulfate outgo from the heavy CaO was more than twice that of the initial year.

A decided decrease in  $\text{Ca(OH)}_2$  concentrations of leachings was also found. Nevertheless, a large fraction of the applied sulfate was still held back by the maximum  $\text{CaO}$  addition.

TABLE 3

*Third annual outgo of sulfates from Cherokee sandy loam—with and without sulfur additions—as influenced by calcic and magnesian materials\**

TANK NUMBER	TREATMENTS PER 2,000,000 LBS. OF SOIL			TOTAL LEACHINGS	AVERAGE OF BaSO <sub>4</sub> PER LITER FROM PERIODIC DETERMINATIONS	S LOST IN LEACHINGS FROM AN ACRE SURFACE EXPOSURE, 2,000,000 LBS. OF SOIL							
	Calcic-Magnesian		Equivalence of 1000 lbs. of S			Oct. 14, 1919	Dec. 8, 1919	Feb. 4, 1920	March 13, 1920	April 2, 1920	June 21, 1920†	Total 1919-1920	
	Material	CaO equivalence											
50	None	None	None	liters	gm.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
51	Limestone	2000 lbs.	None	166.9	0.0426	8.4	9.6	8.4	6.5	4.4	6.2	43.5	
52	Dolomite	2000 lbs.	None	164.3	0.0453	8.0	11.2	9.4	6.8	4.4	5.7	45.5	
53	CaO	2000 lbs.	None	170.0	0.0460	10.2	11.4	9.1	7.0	4.1	6.5	47.8	
54	CaO	3750 lbs.	None	164.8	0.0490	10.0	8.7	9.2	7.4	4.8	8.8	48.9	
55	MgO	2000 lbs.	None	165.1	0.0462	9.6	11.0	9.9	6.5	3.6	6.2	46.8	
56	MgO	3750 lbs.	None	165.4	0.0508	10.5	10.7	9.5	7.3	5.0	8.3	51.3	
57	None	None	FeSO <sub>4</sub>	165.2	0.1086	16.1	29.7	22.2	15.3	12.9	13.1	109.3	
58	CaO	3750 lbs.	FeSO <sub>4</sub>	164.1	0.0745	17.0	18.5	13.3	9.1	7.5	8.8	74.2	
59	MgO	3750 lbs.	FeSO <sub>4</sub>	160.3	0.0632	17.6	7.8	12.2	8.3	6.2	8.4	60.5	
60	CaO	32 tons	FeSO <sub>4</sub>	127.6	0.3637	68.0	66.8	39.2	29.0	22.0	35.7	260.7	
61	MgO	32 tons	FeSO <sub>4</sub>	136.4	0.0636	5.8	13.4	12.0	10.6	8.6	4.1	54.5	
62	None	None	Pyrite	162.8	0.2065	42.5	64.2	36.6	18.8	20.7	23.2	206.0	
63	CaO	3750 lbs.	Pyrite	164.7	0.1524	41.9	44.9	22.7	13.1	10.5	20.4	153.5	
64	MgO	3750 lbs.	Pyrite	165.3	0.1327	45.8	40.4	18.5	10.0	6.4	12.2	133.3	
65	CaO	32 tons	Pyrite	139.0	0.1216	21.0	20.4	14.3	12.4	9.8	18.6	96.5	
66	MgO	32 tons	Pyrite	139.5	0.0932	12.8	25.0	16.8	10.9	7.3	5.8	78.6	
67	None	None	Sulfur	167.2	0.1149	20.2	32.6	22.8	13.6	12.7	15.6	117.5	
68	CaO	3750 lbs.	Sulfur	160.7	0.0797	17.0	21.5	15.0	9.3	7.4	7.9	78.1	
69	MgO	3750 lbs.	Sulfur	162.1	0.0712	17.8	17.9	12.9	8.3	5.5	8.0	70.4	
70	CaO	32 tons	Sulfur	145.1	0.3175	73.9	65.8	30.4	23.7	21.7	47.6	263.1	
71	MgO	32 tons	Sulfur	143.1	0.1335	30.9	37.0	22.7	13.3	8.1	5.4	117.4	

\* Rainfall—August to August 54.75 inches—S precipitated, 44.1 pounds.

† No leaching between June 21 and close of annual period August 4.

*Pyrite additions.* The same order of sulfur recoveries obtained as for the previous year. Slight retardation in oxidation was evidenced by the sulfate outgo from the two oxides in light applications, with more decided indication

in the heavy MgO treatment and still greater repression for the heavy addition of burnt lime. The yield of 67.1 pounds in this last instance, however, was more than double that from the same tank during the previous year. Very much heavier recoveries were obtained from the heavy CaO treatment where sulfur was supplied as sulfates and where derived from oxidation of added elementary sulfur. Since the recovery of added sulfate was increased by light treatments of both CaO and MgO, it is apparent that the oxidation of the pyrite was not accelerated by the light additions of either lime or magnesia, while it was distinctly depressed by the heavier treatments of both materials.

*Sulfur additions.* The sulfate recovery from each of the light additions was greater than that from the control, although the latter contained a considerably larger residue as a result of the greater losses from the CaO and MgO during the first year. All of the treatments yielded greater quantities of sulfates than those which came from the same treatments in the  $\text{FeSO}_4$  group during the second year. But, even with smaller unoxidized residues from additions, the yield from each treatment in the sulfur group was more than that from the corresponding alkali-earth supplement in the pyrite group. Coincident with removal of excess of hydrate through leaching and through carbonation, the repressive influence of the heavy CaO addition was materially lessened. The large outgo of 607.5 pounds of sulfate sulfur from the heavy MgO treatment is accounted for by the fact that the sulfate content of the first leaching of the second year was about equivalent to the aggregate sulfate outgo of the first year and about two-thirds of the total outgo of the second year. As stated, this initial leaching of the second year came after an unusually dry spell, which extended from August to December.

#### OUTGO OF SULFATE DURING THE THIRD YEAR

*No-sulfur additions.* The sulfates passing from the control slightly exceeded the losses from the limestone and dolomite treatments and were almost equal to those from the 1-ton and 3750-pound additions of CaO and MgO, and for the first time equaled the amount brought down by rain.

*$\text{FeSO}_4$  additions.* The leachings from the  $\text{FeSO}_4$  control carried more sulfates than did those of either of the light additions, and also more than those of the heavy MgO treatment. The outgo from the heavy lime incorporation was the maximum for the group and the largest of the progressive increases in annual losses from the same tank during the first three years. The parallel of  $\text{Ca(OH)}_2$  reversion to carbonate and the progressive tendency of the soil to release added, or engendered, sulfates is again indicated. After recovery in excess of the addition, as a result of the extensive leaching of the first year, and the considerable yield for the second year, the heavy MgO dropped during this year to an outgo close to that of the control. Because of the readiness with which the magnesium salts are removed by leaching, it is probable that the sulfate outgo from the heavy addition of magnesium oxide was derived mainly

from the sulfate addition and rainfall rather than from sulfates engendered from components native to the soil.

TABLE 4

*Fourth annual outgo of sulfates from Cherokee sandy loam—with and without sulfur additions—as influenced by calcic and magnesian materials.\**

TANK NUMBER	TREATMENTS PER 2,000,000 LBS. OF SOIL			TOTAL LEACHINGS	AVERAGE OF BaSO <sub>4</sub> PER LITER FROM PERIODIC DETERMINATIONS	S LOST IN LEACHINGS FROM AN ACRE SURFACE EXPOSURE, 2,000,000 LBS. OF SOIL							
	Calcic-magnesian		Equivalence of 1000 lbs. of S			Aug. 14, 1920	Sept. 10, 1920	Dec. 8, 1920	Jan. 10, 1921	Feb. 10, 1921	April 23, 1921	Aug. 4, 1921	Total 1920-1921
	Material	CaO equivalence											
					gm.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
50	None	None	None	187.8	0.0357	4.7	6.9	6.3	6.5	6.2	5.2	4.7	40.5
51	Lime-stone	2000 lbs.	None	192.1	0.0349	5.6	6.8	5.1	5.1	5.6	5.8	6.7	40.7
52	Dolo-mite	2000 lbs.	None	193.5	0.0349	5.2	6.3	5.6	5.2	5.7	6.6	6.3	40.9
53	CaO	2000 lbs.	None	190.1	0.0393	7.7	7.5	5.2	5.4	6.2	6.5	6.6	45.1
54	CaO	3750 lbs.	None	178.3	0.0379	7.3	5.0	4.4	5.0	6.4	5.7	6.4	40.2
55	MgO	2000 lbs.	None	182.1	0.0386	8.0	7.3	5.4	5.0	5.9	5.6	5.5	42.7
56	MgO	3750 lbs.	None	181.0	0.0400	6.6	5.3	6.0	5.1	5.6	6.4	8.0	43.0
57	None	None	FeSO <sub>4</sub>	176.5	0.0626	9.6	14.3	12.0	8.8	10.1	7.8	5.1	67.7
58	CaO	3750 lbs.	FeSO <sub>4</sub>	178.9	0.0451	11.3	10.8	7.3	5.6	5.2	5.0	5.0	50.2
59	MgO	3750 lbs.	FeSO <sub>4</sub>	175.5	0.0429	10.1	9.3	5.5	5.2	5.9	4.9	5.1	46.0
60	CaO	32 tons	FeSO <sub>4</sub>	120.5	0.3232	107.9	42.5	26.9	†	23.0	21.1	†	221.4
61	MgO	32 tons	FeSO <sub>4</sub>	165.8	0.0400	6.7	6.8	7.5	6.3	5.7	4.9	3.5	41.4
62	None	None	Pyrite	178.2	0.1055	16.6	23.9	22.3	15.3	17.2	12.5	8.2	116.0
63	CaO	3750 lbs.	Pyrite	180.8	0.1106	25.2	16.3	16.7	12.9	12.7	14.8	21.1	119.7
64	MgO	3750 lbs.	Pyrite	180.4	0.0772	17.7	17.3	12.2	8.1	18.7	7.8	12.5	94.3
65	CaO	32 tons	Pyrite	156.1	0.1507	29.3	15.8	19.3	11.8	12.2	11.0	32.5	131.9
66	MgO	32 tons	Pyrite	163.6	0.0539	9.4	10.4	10.6	9.1	6.5	4.6	3.7	54.3
67	None	None	Sulfur	180.1	0.0633	8.6	16.3	13.5	8.5	10.8	8.4	3.9	70.0
68	CaO	3750 lbs.	Sulfur	176.4	0.0466	12.3	11.4	6.8	5.2	6.1	5.4	3.9	51.1
69	MgO	3750 lbs.	Sulfur	176.4	0.0459	10.1	8.7	7.2	6.2	6.3	5.5	5.6	49.6
70	CaO	32 tons	Sulfur	164.5	0.2344	57.4	26.7	29.5	18.5	16.5	20.4	48.7	217.7
71	MgO	32 tons	Sulfur	161.4	0.0524	7.3	9.5	11.2	8.5	6.6	4.7	3.3	51.1

\* Rainfall, August to August 55.19 inches; S precipitated, 44.0 pounds.

† No leaching.

*Pyrite additions.* The pyrite control continued to liberate more sulfates to the leachings than did either of the light amendments. The heavy additions of both lime and magnesia still further depressed the sulfate liberation, the

magnesia being the more depressive for both rates. It is apparent that the larger amount of magnesia is very much more depressive upon the agencies responsible for the oxidation of pyrite than upon those responsible for the conversion of elementary sulfur into sulfates.

*Sulfur additions.* After exceeding the control in sulfate losses during the first two years, both light basic treatments trailed in outgo of sulfates during the third year. At the beginning of the third year the heavy MgO treatment had already caused a sulfate sulfur outgo in excess of the amount of elementary sulfur added. The sulfate leaching for the third year, therefore, suffered a decided decrease, though it was still equal to the loss from the sulfur control. It was also  $2\frac{1}{2}$  times as great as the outgo from the no-treatment control and more than twice as great as rainfall sulfur. The heavy line treatment acted as a deterrent, as it did when used with the soluble  $\text{FeSO}_4$ . After progressive increases the maximum outgo occurred during the third year.

Although inhibitive to nitrification during the period of the first four leachings of the initial year, the 32-ton lime treatment distinctly accelerated the nitrate outgo during the leachings of the fifth collection and it has continued to do so during the rest of the five years. Therefore, without amplification of these results, it is not proved that sulfates were not formed biologically from the elementary sulfur added with the heavy treatment of lime, even though such sulfates did not appear in the leachings in amounts so large as those in other treatments of the same group.

#### OUTGO OF SULFATES DURING THE FOURTH YEAR

*No-sulfur additions.* The sulfate loss from the control was almost identical with losses from both native carbonates and the loss from the 3750-pound CaO treatment. It was but little less than the losses from the three other treatments of lime and magnesia in this group.

*$\text{FeSO}_4$  additions.* The outgo from this control continued in the direction of progressive decrease from the maximum of the initial year. The light line treatment and both light and heavy magnesia additions showed the same tendency. The recovery from each of these three treatments was less than the loss from the control, as had been the case during the second and third years, following excessive amounts of the first year. The outgo from the heavy CaO was very much greater than that from any of the other four tanks of the groups. The peak of the sulfate outgo from the heavy lime addition had been reached, however, during the previous year and the loss during the fourth year represents an intermediate step between the outgo of the third year and that of the fifth year, in progressive recession from the maximum.

*Pyrite additions.* The recovery from the control and that from the light CaO supplement were in close agreement. Both recoveries showed positive progressive decrease from corresponding maximum losses of the second year. The same was true of the somewhat smaller outgo from the 3750-pound

MgO addition. A still smaller recovery, the smallest of the pyrite group, came from the heavy MgO treatment, and maintained the progressive order of decrease from the maximum of the initial year. This order of outgo was the same as that maintained by the added soluble sulfate. The largest recovery

TABLE 5  
Fifth annual outgo of sulfates from Cherokee sandy loam—with and without sulfur additions—as influenced by calcic acid and magnesian materials\*

TANK NUMBER	TREATMENTS PER 2,000-000 LBS. OF SOIL			TOTAL LEACHINGS	AVERAGE OF BaSO <sub>4</sub> PER LITER FROM PERIODIC DETERMINATIONS	S LOST IN LEACHINGS FROM AN ACRE SURFACE EXPOSURE, 2,000,000 LBS. OF SOIL					
	Calcic-magnesian		Equivalence of 1000 lbs. of S			Dec. 20, 1921	Jan. 21, 1922	Mar. 2, 1922	April 28, 1922	Aug. 3, 1922	Total 1921-1922
	Material	CaO equivalent lbs. or tons									
50	None	None	None	142.3	0.0386	5.5	8.1	8.3	7.0	4.6	33.5
51	Limestone	2000 lbs.	None	139.2	0.0415	8.6	9.1	5.5	6.1	5.5	34.8
52	Dolomite	2000 lbs.	None	139.2	0.0444	8.6	10.0	7.9	5.9	4.8	37.2
53	CaO	2000 lbs.	None	133.2	0.0429	8.3	7.8	7.4	5.9	4.8	34.2
54	CaO	3750 lbs.	None	132.7	0.0437	10.1	7.4	6.8	5.0	5.4	34.7
55	MgO	2000 lbs.	None	135.0	0.0429	9.2	8.4	6.7	5.9	4.7	34.9
56	MgO	3750 lbs.	None	138.4	0.0408	8.9	6.4	7.6	5.6	5.3	33.8
57	None	None	FeSO <sub>4</sub>	124.6	0.0560	5.8	9.2	10.1	9.8	7.5	42.4
58	CaO	3750 lbs.	FeSO <sub>4</sub>	130.2	0.0473	8.2	8.9	8.0	6.2	6.2	37.5
59	MgO	3750 lbs.	FeSO <sub>4</sub>	128.6	0.0473	8.4	9.1	7.2	6.3	5.5	36.5
60	CaO	32 tons	FeSO <sub>4</sub>	110.9	0.2402	95.2	19.4	15.7	10.5	6.4	147.2
61	MgO	32 tons	FeSO <sub>4</sub>	107.1	0.0510	4.3	8.8	11.4	6.6	3.2	34.3
62	None	None	Pyrite	132.3	0.1077	18.7	18.8	19.8	16.8	12.5	86.6
63	CaO	3750 lbs.	Pyrite	132.4	0.1136	29.1	20.0	15.1	13.7	13.9	91.8
64	MgO	3750 lbs.	Pyrite	133.4	0.0699	18.7	12.2	8.4	8.5	7.9	55.7
65	CaO	32 tons	Pyrite	108.6	0.1405	38.8	14.3	11.4	8.2	14.0	86.7
66	MgO	32 tons	Pyrite	111.2	0.0713	8.7	14.4	14.8	7.8	4.2	49.9
67	None	None	Sulfur	137.5	0.0611	8.9	11.6	11.5	10.7	7.6	50.3
68	CaO	3750 lbs.	Sulfur	132.7	0.0473	8.8	9.4	7.7	6.6	5.3	37.8
69	MgO	3750 lbs.	Sulfur	134.4	0.0459	9.8	9.7	6.9	5.4	5.2	37.0
70	CaO	32 tons	Sulfur	112.2	0.1827	76.2	17.3	9.6	6.3	7.6	117.0
71	MgO	32 tons	Sulfur	104.5	0.0575	5.7	9.8	11.5	6.9	3.5	37.4

\* Rainfall, August to August 56.75 inches; S precipitated 38.0 pounds.

came from the heavy CaO admixture and represented the maximum up to this time in the progressive increases from the minimum of the initial year.

*Sulfur additions.* The sulfate recoveries from the three additions, light CaO and both light and heavy MgO, were practically identical and consider-



ably less than the outgo from the control. The two lighter treatments and the control gave up sulfates in amounts which placed them in the position of showing the third successive drop in the progressive decrease from maxima of the initial year, being parallel in this respect to their effect when used in connection with the additions of the soluble sulfate. The heavy MgO gave a sulfate return in similar progressive decrease from the maximum of the second year. The maximum for this treatment came in the second year rather than in the first, because of the previously mentioned repressed leaching as a result of marked influence upon the physical characteristics of the soil. The heaviest yield of the year came from the burnt lime, which was also the case in the other two groups which received sulfur carriers. This was the second largest recovery of the 5-year period, corresponding in that regard to the return induced by the same treatment when used in conjunction with the soluble sulfate of iron.

#### OUTGO OF SULFATES DURING THE FIFTH YEAR

*No-sulfur additions.* The losses from the control, the two lime, the two magnesia, and the two limestone additions were in close agreement, and each outgo was less than the amount of sulfate sulfur brought down by rainfall. These concordant losses corresponded in general to those from the soluble sulfate additions and those from the elementary sulfur additions, except where the heavy amount of lime was added to the two forms. They were still appreciably less, however, than the several recoveries from the pyrite for the fifth year.

*FeSO<sub>4</sub> additions.* The amounts of sulfate sulfur given up by the light lime and both light and heavy MgO were nearly the same and somewhat less than the amount derived from the ferrous-sulfate control. Each yield represented, respectively, the minimum outgo from the several joint treatments over the 5-year period. The leachings from the heavy addition of burnt lime carried the largest sulfate yield derived during the year from the iron sulfate group. This outgo of 147.2 pounds of marked the second drop in annual outgo from the 260.7-pound maximum of the third year.

*Pyrite additions.* As compared with the several recoveries of the preceding year, all pyrite additions showed decreases in outgo. With the exception of that from the heavy burnt lime, each recovery for the year represented the minimum outgo for the 5-year period. The sulfates released to the leachings by the 32-ton burnt-lime addition constituted the heaviest return from the sulfur group during the year.

*Sulfur additions.* In harmony with the recoveries from the sulfate group, those from the two light oxide additions and the 32-ton treatment were practically identical, equivalent to rainfall sulfates and less than the recovery from the elementary sulfur control. Each of these four recoveries represented, respectively, the minimum annual outgo from the several combinations for the 5-year period. The heavy CaO gave the largest recovery of the sulfur group

for this year. However, as was true also of the sulfate, group, the fifth annual outgo from the heavy burnt lime was next to the smallest of the 5-year period. In the case of the heavy CaO supplements, both groups showed the decreasing order in outgo for third, fourth, second, fifth, and first years.

TABLE 6

Summary of leaching data from 256 inches precipitation for the 5-year period and calculation of total loss in per cent of sulfur content of soil, sulfur additions and rainfall

TANK NUMBER	TREATMENT PER 2,000,000 LBS. OF SOIL			LEACHING DATA FOR 5-YEAR PERIOD							
	Calcic — magnesian		Equivalent of 1000 lbs. of S	Total volume leached	Average BaSO <sub>4</sub> per liter	Total sulfate-sulfur outgo as					
	Material	CaO equivalent lbs. or tons				S per 2,000,000 lbs. of soil	Fraction of initial sulfur content of soil	Fraction of sulfur applied	Related to sulfur in rainfall	Excess per acre	
										Above S in rainfall	Above loss from no-treatment tank
				liters	gm.	lbs.	per cent	per cent	per cent	lbs.	lbs.
50	None	None	None	716.4	0.0394	175.8	10.8		81.9	—38.9	
51	Limestone	2000 lbs.	None	713.8	0.0599	321.9	14.2		108.0	17.2	56.1
52	Dolomite	2000 lbs.	None	713.2	0.0629	241.8	14.8		112.6	27.1	66.0
53	CaO	2000 lbs.	None	709.0	0.0642	247.8	15.2		115.4	33.1	72.0
54	CaO	3750 lbs.	None	674.3	0.0769	260.6	16.0		121.4	45.9	84.8
55	MgO	2000 lbs.	None	690.9	0.0670	240.5	14.8		112.0	25.8	64.7
56	MgO	3750 lbs.	None	689.3	0.0795	278.5	17.1		129.7	63.8	102.7
57	None	None	FeSO <sub>4</sub>	665.8	0.3512	934.6	57.3	93.5	435.3	719.9	758.8
58	CaO	3750 lbs.	FeSO <sub>4</sub>	668.7	0.4548	1164.4	71.4	116.4	542.3	949.7	988.6
59	MgO	3750 lbs.	FeSO <sub>4</sub>	653.9	0.6156	1196.1	73.4	119.6	557.1	981.4	1020.3
60	CaO	32 tons	FeSO <sub>4</sub>	546.1	0.2771	889.9	54.6	89.0	414.5	675.2	714.1
61	MgO	32 tons	FeSO <sub>4</sub>	566.4	0.7364	1321.2	81.1	132.1	615.3	1106.5	1145.4
62	None	None	Pyrite	681.4	0.2253	884.7	54.3	88.5	412.1	670.0	708.9
63	CaO	3750 lbs.	Pyrite	681.4	0.2181	817.4	50.1	81.7	385.2	602.7	641.6
64	MgO	3750 lbs.	Pyrite	687.0	0.1982	721.5	44.3	72.2	336.1	506.8	545.7
65	CaO	32 tons	Pyrite	620.1	0.1106	413.7	25.4	41.4	192.7	199.0	237.9
66	MgO	32 tons	Pyrite	583.8	0.1751	455.5	27.9	45.6	212.2	240.8	279.7
67	None	None	Sulfur	697.1	0.3080	955.6	58.6	95.6	445.1	740.9	779.8
68	CaO	3750 lbs.	Sulfur	666.2	0.4227	1136.2	69.7	113.6	529.2	921.5	960.4
69	MgO	3750 lbs.	Sulfur	675.8	0.4280	1192.2	73.1	119.2	555.3	977.5	1016.4
70	CaO	32 tons	Sulfur	633.2	0.2331	891.6	54.7	89.2	415.2	676.9	715.8
71	MgO	32 tons	Sulfur	567.4	0.8367	1239.2	76.0	123.9	577.2	1024.5	1063.4

## TOTAL SULFATE LOSSES DURING THE 5-YEAR PERIOD

Table 6 gives the totals of leaching volumes and sulfur losses for the 5-year period and the grand averages of  $\text{BaSO}_4$ -per-liter determinations.

*Volumes of leachings.* Space forbids a full discussion of the influence of treatment upon the volumes of leachings from rainfall, for such a discussion would involve a detailed consideration of variation in soil bulk due to treatment and of periodicity of precipitation. It is sufficient to point out one or two relationships. The differences between the leaching from the control and the leachings from the two limestone additions are negligible, since no secondary effect of residues from enhanced plant growth is involved. Both lime treatments and both magnesia additions, however, yielded leaching volumes somewhat smaller than those of the control. All of the  $\text{FeSO}_4$  additions gave collections considerably below those of the control and also light amendment treatments. Considerable diminution in leaching volumes was caused by the heavy additions used in connection with the three sulfur carriers. This effect was probably due in part to the larger resultant mass, as well as to amelioration in physical condition. In spite of decreased leaching volumes, the large applications of burnt lime produced a seemingly dry granular soil which must have retained far more moisture than that indicated by its physical condition. The magnesia, however, produced a somewhat sticky condition and the soil apparently retained the excess of moisture in a different manner than that treated with burnt-lime.

The data in the sixth column of table 6 show the average of  $\text{BaSO}_4$  determinations on the liter basis, from which may be seen the material analytical differences obtained.

*No-sulfur additions.* The aggregate loss of sulfates from the control was equivalent to 10.8 per cent of the soil's initial content of sulfur. There was a conservation of 38.9 pounds, or 18.1 per cent, of the precipitated sulfates for the 5-year period. The passage of rainfall sulfur through this soil amounted to 81.9 per cent of the precipitation, as compared with 69 per cent in the case of the control of the previously reported 8-year experiment.

The total losses from the limestone and dolomite treatments were rather close, the dolomite having given an average annual yield of 2 pounds more than the limestone. The 10-pound excess for the period was equivalent to about one-third of the amount of sulfur carried by the sphalerite or other sulfur materials in the dolomite. The differences between sulfate sulfur leachings and the total 5-year precipitation of sulfate sulfur—214.7 pounds—give total actual sulfate losses of 17.2, 27.1, 33.1, 45.9, 25.8, and 63.8 pounds, respectively, for tanks 51 to 56 inclusive. These excesses of outgo above sulfur income are equivalent, respectively, to annual losses of 3.4, 5.4, 6.6, 9.2, 5.2, and 12.8 pounds. The 6 tanks 51–56 show losses in excess of the control outgo varying from 56.1 pounds to 102.7 pounds.

*FeSO<sub>4</sub> additions.* The addition of soluble ferrous sulfate, unsupplemented, gave a recovery of 934.6 pounds of sulfate sulfur from the 1000-pound application. When the rainfall sulfur is subtracted from this, a recovery of 719.9 pounds, or 71.99 per cent, of the sulfate treatment is accounted for. The difference between the outgo from the FeSO<sub>4</sub> and no-treatment controls gives an actual yield of 758.8 pounds. Since the recovery of the fifth year was only 50.3 pounds, as against a 33.5-pound loss from the control and 38.0 pounds from rainfall it is most probable that years will be required to equalize the disparity of 280.1 pounds if such is still in the acid soil.

It was interesting to observe that no ferrous sulfate ever appeared in the leachings, every one of which was free of iron and alkaline to methyl orange. The FeSO<sub>4</sub> was neutralized by the silicates of the acid soil, for increase in sulfate content of the leachings was accounted for in the main by increases in calcium and magnesium content. This is not in harmony with the viewpoint of Van Bemmelen (9), cited by Rudolfs (8), to the effect that sulfuric acid generated in the soil is more active upon the iron oxide than upon silicates of the soil, as set forth by the quotation "the iron oxide in the soil protects, to a certain extent, the humate and silicate bases against the action of sulfuric acid.

Assuming continued occurrence of the unrecovered fraction of the applied soluble sulfate it seems hardly probable that this unaccounted-for difference could be held in original form or as basic sulfates in the *acid* soil with such tenacity as to resist the solvent action of the repeated leachings. But the factors of ample drainage and optimum aeration do not favor the assumption of de-sulfonation. However, this alternative finds some support from the fact that the acid soil control gave a decidedly lower recovery than was obtained from the alkaline conditions other than that induced by the heavy burnt lime. It is possible, however, that some of the soluble added sulfates may have resulted in the formation of insoluble sulfates in the soil.

The 1750-pound excesses above the 2000-pound alkali-earth treatments intended to insure the immediate conversion of the acid sulfate to calcium or magnesium sulfate, were responsible for decided increases in leached sulfates. The two treatments of CaO and MgO were responsible, respectively for sulfur yields of 1164.4 pounds and 1196.1 pounds, or recoveries of 94.97 per cent and 98.14 per cent, after deduction of the increment from rain water and corresponding yields of 988.6 pounds and 1020.3 pounds after deducting the outgo from the untreated control. The heavy addition of MgO was responsible for a still greater and a much quicker passage of sulfates. Of the total of 1321.2 pounds for the 5-year period 1007.1 pounds were recovered from the five leachings of the first year and 183.9 pounds from the six leachings of the second year. After deduction of the income from rainfall sulfur, an outgo of 106.5 pounds in excess of the amount added was obtained. Deducting the 175.8-pound loss from the no-treatment control, an actual outgo of 1145.4 pounds, or 145.4 pounds in excess of addition was obtained. These excesses

necessarily were derived from the sulfur content of the soil or in part from alkali-earth-treatment impurities.

The heavy burnt-lime treatment exhibited a distinct retardation in the outward movement of sulfates. This repressive tendency was most effective

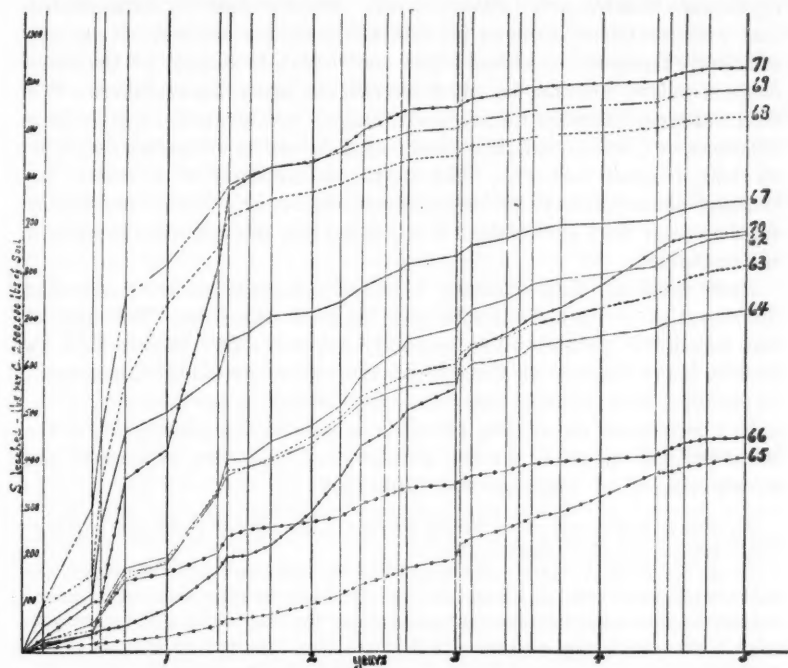


FIG. 2. INFLUENCE OF CaO AND MgO UPON LEACHINGS OF SULFATES DERIVED FROM PYRITE AND FLOWERS OF SULFUR, AS DETERMINED FROM PERIODIC LEACHINGS OVER A 5-YEAR INTERVAL

Pyrite		Sulfur	
Tank 62.....	———— No supplement	Tank 67.....	————
Tank 63.....	----- CaO, 2,000 lbs. (+ 1750)	Tank 68.....	-----
Tank 64.....	- - - - MgO, 2,000 lb. CaO equivalent (+ 1750)	Tank 69.....	- - - -
Tank 65.....	x-x-x-x-x CaO, 32 tons	Tank 70.....	x-x-x-x
Tank 66.....	o-o-o-o-o MgO, 32-ton CaO equivalent	Tank 71.....	o-o-o-o-o

during the first year. The outgo for the second year was more than twice that of the initial year, while the maximum annual leaching came during the third year. Each of the five leachings of the first year contained  $\text{Ca}(\text{OH})_2$ , the average normality having been 0.00654, within a range between a maximum of

0.0158 normal to a minimum of 0.0005 normal. The same held true for the first three of the five leachings of the second year, which showed an average normality of 0.00157, with extremes of 0.00225 and 0.0008. Even the third, fourth, and fifth of the six leachings of the third year showed alkalinity ranging between 0.000075 and 0.000425 normal. Previous data (6) demonstrated that with conditions optimum for saturation, these concentrations are not sufficiently repressive in solvent action upon  $\text{CaSO}_4$  to account for the lesser outgo of sulfates from the soil which received the heavy lime additions. The  $\text{CaO}$  treatment has either transformed the added sulfate into less soluble combinations, or it has so materially ameliorated the soil as to increase decidedly its ability to retain the  $\text{CaSO}_4$ , which must have been formed as a result of the thorough dissemination of the large excess of alkali-earth. From considerable supplementary work already done it is believed that this phase of the problem will be clarified.

*Pyrite additions.* It is commonly observed that pyrite oxidizes during the disintegration of exposed minerals carrying it as impurities. The mineral was included in parallel with elementary sulfur in order to determine its activity in the soil, with the thought that the sulfates derived therefrom would be furnished more gradually than from sulfur "flowers" or powder.

In a preliminary report upon related laboratory studies growing out of the use of pyrite in the soil of the 1917 installation of lysimeters, reference to the sulfate content of leachings was made (3):

The data secured demonstrated that the flowers of sulfur and iron pyrite were both converted into sulfates with distinct rapidity.

It was first assumed that the oxidation of both the elementary sulfur and that of the pyrites was induced in the main, if not solely, by organisms. However, some doubt concerning this assumption was introduced about 2 yrs. after the inauguration of the experiment, when it was observed that a strong odor of sulfur dioxide was given off from the reserve sample of iron pyrites, which had been kept in the dark in an 8-oz. glass bottle, tightly stoppered with an ordinary No. 6 cork stopper. A 10-g. charge of the pyrites was found to yield soluble sulfate of iron equivalent to a determined weight of 0.4172 g. of  $\text{BaSO}_4$ , as an average of seven determinations. The same observation has been reported by Allen and Johnston.

These investigators accounted for the oxidation by means of the equation:



They also found that the grinding of pyrite and marcasite for 1-hour and 6-hour periods was responsible for decided increases in sulfate generation. The rapidity and extent of this result were so marked as to preclude the probability of bacterial activities, as against the probability of chemical activation through frictional heat and increase of surface. In September, 1919, Mr. F. J. Gray formerly of this laboratory staff, conducted an adjunct laboratory experiment, using the same soil, materials, and amounts, in an effort to differentiate between chemical and biochemical agencies responsible for the oxidation of pyrite and also elementary sulfur under dry and optimum moisture conditions. These results have been held for publication, as supplementary to



those of the parent lysimeter investigation. They show that the oxidation is brought about by both chemical and biochemical agencies.

The sulfate leachings are of interest in their connection with the results of Rudolfs (7), who has recently reported studies which dealt with the activities of bacteria in oxidizing pyrite. In the paper upon bacterial oxidation of pyrite no mention was made by Rudolfs of our related lysimeter results (3, 4) reported under captions which referred only to elementary sulfur. In a later contribution Rudolfs (8) referred to one of the two preliminary papers (4), as to chemical oxidation of elementary sulfur, but apparently overlooked our assumption that bacterial activities to some extent accounted for the recovery of sulfates in the leachings from pyrite.

While it is undoubtedly true, as pointed out by Rudolfs (7), that bacteria can and do convert the  $\text{FeS}_2$  of pyrite into sulfates in soil media, it is also apparent that the inherent oxidative properties and moisture conditions of the soil serve to accelerate the purely chemical oxidative reactions which transpire even in air-dry condition in the atmosphere.

The sulfur recoveries from the pyrite group do not show the same order which obtained in the ferrous sulfate group. The maximum sulfur recovery, as sulfates leached from the pyrite additions, was obtained from the acid soil, in which respect this control differed from the controls of the other two sulfur-addition groups. A distinct retardation was caused by the light lime treatment, while the light  $\text{MgO}$  addition was even more effective in the same direction. This relationship between the three totals for the 5-year period was consistently maintained between the respective annual losses of the first three years, which gave the three largest losses in each case, the outgo of the second year having been the maximum for each of the three conditions. The heavy additions of  $\text{CaO}$  and  $\text{MgO}$  were both still more decidedly depressive upon sulfate generation, if the repressive influence of outgo and depressed generation be considered as identical. It will be observed that the heavy  $\text{CaO}$  addition occupied the same relative position, that of minimum, as in both the  $\text{FeSO}_4$  and elementary sulfur groups. This was not the case, however, with the  $\text{MgO}$ , which caused the maximum sulfate outgo from the sulfur additions of the other two groups. Furthermore, the heavy burnt-lime addition increased progressively from the minimum of the initial year to the maximum of the fourth year. This progressive increase followed a progressive decrease in  $\text{Ca(OH)}_2$  concentrations in the leachings. Calcium hydroxide was present in all of the five collections of the first year, in all five of those of the second year, and in four of the six of the third year. The average of  $\text{Ca(OH)}_2$  concentrations of leachings for the three years were 0.01327 N., 0.001255 N., and 0.00055 N., in order. On the other hand, the corresponding  $\text{MgO}$  addition gave its maximum sulfate outgo during the first year, with progressive decreases to its minimum in the fifth year. In this regard, the heavy  $\text{MgO}$  treatment acted as it did in the other two groups. The heavy lime treatment also showed somewhat the same tendency as in the other two

sulfur-addition groups. It is apparent that the influences of the excessive additions of both oxides were very much more depressive to the chemical or biochemical, or both, reactions responsible for the oxidation of  $\text{FeS}_2$ , than to the reactions which convert sulfur into sulfates. However, even assuming that all precipitated sulfates were leached through the soil, deducting such from the total leachings, and assuming no sulfates to have been derived from the organic matter of the soil or basic treatment, we find that 67.0 per cent of the added  $\text{FeS}_2$  has been converted into sulfates in the acid control. With the same assumptions, we find recoveries of 60.3 per cent and 50.7 per cent from the light treatments of  $\text{CaO}$  and  $\text{MgO}$ , respectively, and 19.9 per cent and 24.1 per cent from the two heavy additions. Thus, in the case of the lighter treatments,  $\text{MgO}$  was more retardative than  $\text{CaO}$  upon the outgo of sulfates, while the reverse was true for the heavy additions. Applying the 175.8-pound outgo from the untreated control as a corrective factor to the sulfate leachings from the five pyrite tanks, 64.16 per cent and 54.57 per cent recoveries came from light  $\text{CaO}$  and  $\text{MgO}$ , respectively, as compared with 70.89 per cent from the sulfate control, while the two heavier oxide treatments gave corresponding recoveries of only 23.79 per cent and 27.97 per cent.

*Sulfur additions.* The sulfate yields from the elementary sulfur group occurred in the same order as those of the  $\text{FeSO}_4$  group. In both cases maximum recovery came from the heavy  $\text{MgO}$  addition and the minimum from the heavy burnt-lime treatment. More than one-half of the total recovery from the sulfur control was obtained in the leachings of the first year, while still larger fractions came from the light treatments of  $\text{CaO}$  and  $\text{MgO}$ . The recovery from the 1000-pound-S-addition control during the first two years amounted to 75.1 per cent. The recoveries from the sulfur treatment and the two supplementary  $\text{CaO}$  and  $\text{MgO}$  treatments at the 2000-pound-plus rate, and the  $\text{MgO}$  treatment at the 32-ton rate during the same 2-year period, were 85.3 86.8 and 83.4 per cent, respectively.

Only about 10 per cent of the total recovery from the heavy burnt-lime treatment was found in the leachings of the first year. This small outgo of 88.6 pounds was a little more than twice the amount of sulfur precipitated in rain water during 1917, and nearly five times the outgo from the untreated control. It was followed by a material increase during the second year and a still greater increase to the maximum annual outgo during the third year. In this respect it was parallel with nitrate outgo. The average concentrations for those leachings carrying  $\text{Ca(OH)}_2$  during the first three years were 0.012719 N., 0.001564 N., and 0.000633 N., as obtained, respectively, from hydroxide occurrences in four of the five leachings of the first year, four of the five of the second year, and three of the six of the third year. The fact that after rainfall correction, the recovery of sulfur from the 1000-pound addition with 32 tons of  $\text{CaO}$  was only 676.9 pounds cannot be taken as proving the sulfication of only this amount. For, with the same rainfall correction, only 675.2 pounds were recovered from the 1000-pound sulfate sulfur addi-

tion with 32 tons of CaO. These two recoveries are remarkably close, and they might be construed as indicating the complete oxidation, though incomplete recovery, of the added flowers of sulfur.

Assuming complete leaching of rainfall sulfur and no sulfates derived from the supplies native to the soil, or basic treatment, over the 5-year period, the recoveries were 740.9 pounds from the control, 921.5 pounds from the light application of CaO, 977.5 pounds from the light application of MgO, and 676.9 pounds from the heavy burnt-lime addition. Applying the same assumptions to the maximum outgo of 1239.2 pounds from the 32-ton MgO addition, the recovery obtained was 1024.5 pounds or 24.5 pounds more than the addition. It is quite possible, however, if not probable, that some of the elementary sulfur applied to this group has not been converted into sulfates, and that the apparent respective recoveries are attributable in some measure to soil-derived sulfates.

By subtraction of the no treatment outgo from the 1239.2-pound outgo, an increased outgo of 63.4 pounds over the 1000-pound addition is accounted for. The lighter MgO treatment also gave sulfates in excess of the 1175.8-pound total represented by control outgo plus the 1000-pound addition. On this basis the light lime treatment failed of full recovery by 39.6 pounds, while 220.2 pounds and 284.2 pounds remained unrecovered in sulfur control and sulfur plus heavy lime treatment, respectively.

#### RELATION OF OUTGO TO PRECIPITATED SULFUR AND INITIAL SOIL CONTENT

*No-sulfur additions.* This relationship can be considered more directly in the case of no-sulfur additions in tanks 50 to 56, inclusive. Had there been no sulfur added from the atmosphere, the 5-year-period loss from the control would have amounted to 10.8 per cent of the initial sulfur content of the soil. This loss is accounted for by leachings ranging from the minimum of 18 pounds during the initial of subnormal rainfall to the maximum of 46.6 pounds as a result of a 54.75-inch precipitation during the third year. In the case of the leachings from the six alkali-earth additions, however, the losses ranging from 14.2 per cent to 17.1 per cent were the result, in every case, of a maximum during the first year, with progressive decrease to the minimum during the fifth year. These same six treatments gave sulfate losses ranging from 108.0 per cent to 129.7 per cent of the sulfur derived from rainfall. Only the control failed to leach as much sulfur as was derived from this source.

*Additions.* The sulfate recoveries from the added sulfate ranged from amounts equivalent to 54.6 per cent up to 81.1 per cent of the native sulfur content of the soil. The pyrite-addition group gave recoveries ranging from 25.4 per cent to 54.3 per cent of the amount originally contained by the soil. In the case of the elementary sulfur group, the range was from 54.7 per cent to 76.0 per cent. Each sulfur addition gave a recovery largely in excess of the sulfur brought down by rain, the smallest per cent excess of 192.7 came from

the heavy burnt lime and pyrite, while the largest 615.1 came from the heavy application of MgO with  $\text{FeSO}_4$ .

#### SUMMARY

Sulfate analyses of 614 collection from 22 lysimeters over a 5-year period are reported in a study of the loss of native stores of sulfurous materials and recoveries of sulfur derived from rainfall and 1000-lb.-per-acre additions of sulfur as  $\text{FeSO}_4$ , pyrite, and elementary sulfur, as such are influenced by various forms and amounts of calcic and magnesian materials.

The sulfate outgo from the no-treatment tank was less than that brought down by rainfall. Each of the treatments CaO, MgO, limestone, and dolomite at the 2000-lb, CaO-equivalent rate, and CaO and MgO, at the 3750-lb. rate, increased the outgo of sulfate sulfur to amounts somewhat in excess of those of the control and rainfall sulfur.

The unsupplemented  $\text{FeSO}_4$  treatment, and also that with light supplements of both lime and magnesia and heavy supplement of magnesia, gave the largest annual recoveries during the first year. All three supplements also induced recoveries in excess of the recovery from the control, the totals coming in the order named. The heavy burnt lime treatment was initially very depressive to the outgo of the added sulfate radical. This effect was less marked after the first year, but its total recovery for the 5-year period was less than that of the sulfate control. The totals from the two light supplements and heavy magnesia were in excess of the sulfate addition. After deduction of rainfall sulfur, however, only the heavy MgO treatment gave a recovery equivalent to that of the addition.

The largest initial annual recovery and the largest 5-year total from the pyrite additions came from the acid-soil control. Both light and heavy supplements of lime and magnesia were depressive to sulfate leachings from the pyrite, the heavier applications having been decidedly more so than the lighter additions. In this respect the light additions differed from their activities upon  $\text{FeSO}_4$  and sulfur. As in the  $\text{FeSO}_4$  group, the heavy burnt lime was the most depressive of the four treatments.

Both CaO and MgO in light amounts and the heavy treatment of MgO accelerated the outgo of sulfates and increased the respective totals for the 5-year period when used as supplements to the powdered sulfur. The initial depressive influence of the heavy burnt-lime addition diminished after the first year, but its effect was still evidenced in the 5-year total, which was less than that of the control.

In agreement with previous work where no sulfur was added, increase in MgO additions caused increased sulfonation of powdered sulfur and greater recoveries of added soluble sulfate. The lighter magnesia additions to both sulfur and ferrous sulfate caused smaller recoveries than the heavy MgO treatments and greater returns than those from additions of CaO. However,

the light CaO supplements to sulfur and  $\text{FeSO}_4$  produced larger recoveries than were obtained from the respective sulfur and sulfate controls. The lesser recoveries from heavy CaO additions, to both sulfur and  $\text{FeSO}_4$ , were practically identical. It is not proved, therefore, that the minimum recovery from the sulfur group was due to the inhibitory effect of the heavy lime upon oxidation of the added sulfur.

Since the light application of MgO was more depressive than the corresponding CaO treatment to sulfate outgo from pyrite, while the heavy MgO was still more depressive, it is evident that the chemical or biochemical agencies responsible for the conversion of pyrite into sulfates differ from those responsible for the derivation of the same end-products from sulfur. For, both  $\text{FeSO}_4$  and sulfur groups show that  $\text{MgSO}_4$  would have been leached readily had it been present.

Disregarding the increment from rainfall, the recovery from both  $\text{FeSO}_4$  and S controls during the first year was about one-half of that added, while that from pyrite was only about one-fifth of the addition. With the same inconsistency, the  $\text{FeSO}_4$ , sulfur, and pyrite controls had yielded 82, 83, and 68 per cent, respectively, of the sulfur additions after three years of leachings. In the order of light CaO, light MgO, and heavy MgO supplements, both sulfate and sulfur showed recoveries in excess of the 1000-pound additions at the end of the third year. With partial recovery from each pyrite addition, the same amendments induced recoveries in reverse order after three years.

After deducting the income from rainfall, only the recoveries of 1106.5 pounds and 1024.5 pounds induced by MgO from  $\text{FeSO}_4$  and S, respectively, were in excess of the 1000-pound additions.

After deduction of the control sulfate outgo the recoveries from sulfate and sulfur additions with both MgO supplements were in excess of the added sulfur. On the same basis the other recoveries ranged between 237.9 pounds for pyrite and heavy burnt lime, to 988.6 pounds for  $\text{FeSO}_4$  plus light lime treatment.

All 6 tanks treated with lime, or magnesia, without sulfur gave sulfate losses in excess of the amount precipitated during the 5-year period.

The continued depressive action of the heavy CaO treatment and the persistence of  $\text{Ca}(\text{OH})_2$  in the leachings were shown to be correlated. Conversely, carbonation of the excess of solid-phase  $\text{Ca}(\text{OH})_2$  and increase in outgo of sulfates from  $\text{FeSO}_4$  and S additions were found to be parallel. The recovery of the initial depressive effect was much slower, however, in the case of pyrite.

Although the previously noted divergent effect of increasing amounts of CaO and MgO upon native sulfur stores was found to obtain also in the case additions of sulfur as  $\text{FeSO}_4$  and "flowers," convergence was shown in the case of pyrite.

The results have certain practical significance. Economic and equivalent applications of burnt lime, magnesia, limestone, and dolomite were practi-

cally identical in their accelerative influences upon sulfonation of native sulfurous materials. Oxidation of powdered sulfur was increased by small amounts of both lime and magnesia; but, undesirable losses may be caused by injudicious use in amounts which may be considered as within practical range. Pyrite may be considered as a source of sulfur, where less rapid generation is desired, particularly in an acid soil, since both lime and magnesia depress the amounts of water-soluble sulfates engendered. The use of excessive amounts of both CaO and MgO are inadvisable in connection with either sulfur or pyrite.

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# A PRELIMINARY STUDY OF THE EFFECTS OF SODIUM CHLORIDE UPON ALFALFA GROWN IN SOLUTION CULTURES<sup>1</sup>

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Aside from the interest that attaches to the question of the effect of chlorides upon plants because of the fact that some potassic fertilizers come in the form of chlorides or contain varying amounts of sodium chloride, there is also the observation, made by investigators and farmers alike, that some plants are favored while others are harmed when grown in the presence of sodium chloride. Of recent work bearing upon this question, mention should be made of the investigations of Soderbaum (11), Tottingham (14), Krishnamurti Row (7), Trelease (16), Harlow (5), Schneidewind, *et al.* (9), and Guglielmetti (4). From their results it is obvious that different crops will have to be studied independently with respect to the influence exerted upon them by this compound.

The writer was led to take up the study of the effect of sodium chloride upon alfalfa, first, as a result of a previous investigation done jointly with Dr. G. S. Fraps (2), at the Texas Agricultural Experiment Station, in which it was found that alfalfa from various parts of the country differed greatly with respect to chlorine content, varying from about 0.2 per cent to over 1.0 per cent, expressed as sodium chloride. The possibility suggested itself that chlorine or sodium chloride may be of some benefit to this plant. Another reason was the desire to undertake a systematic study of the salt requirements of this plant in nutrient solutions, and it was thought advisable to obtain information with respect to the necessity of including a chloride, more especially sodium chloride, among the salts to be tested.

There is also of course the purely scientific interest in the still moot question as to whether chlorine is essential (as distinguished from advantageous) for the growth of at least some plants.

By way of a suggestive remark it may be pointed out that in view of the fact that in some local areas there exists a special alfalfa problem in the sense that alfalfa land may, for no apparent reason, cease to produce profitable crops, any data concerning the nutritional relations of this plant should be

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welcomed as possibly contributing towards an intelligent investigation of the causes underlying this trouble.

The preference for western over eastern alfalfa in the hay market may possibly be due to a difference in composition, manifesting itself in varying palatability, in which the chlorine content may well play a part.

This study must of necessity be of a preliminary character, since up to the present time comparatively little work, if any, has been done in the way of a systematic study of the salt requirements of alfalfa. There is no such mass of data relating to alfalfa as has already accumulated and is being added to continuously in the case of wheat, soy beans, buckwheat, and other plants. This is rather strange, considered from the standpoint of the economic importance of the alfalfa crop. The reason may be the fact that this plant, on account of its delicate initial growth, does not lend itself so easily to solution culture work as do plants with large seeds or those of a short growing period or of a sturdier habit of growth.

#### OUTLINE OF WORK

A commercial variety of alfalfa obtained from the seed testing laboratory of this station was germinated on blotting paper kept moist with a solution one-tenth the strength of the one used in the check solution culture. It was at first intended to obtain the average weight of single seeds and to use only those that approached very closely this average. But it was soon found that the seeds were very uniform in this respect, showing hardly any significant variation from the 2 mgm. average obtained on weighing a few lots of one hundred seeds each. A fine, even stand was secured after three days, at which time the seedlings were transferred to a germinating net, as described by Shive (10). On the third of January, 1923, when the seedlings reached a length of about 7 cm., they were put into the jars containing the culture solutions, according to the procedure adopted by Tottingham (13) and by Shive (10), using three plants to each jar. The cultures were run in duplicates and two series; A and B, were started at the same time. In series A the sodium chloride was introduced into the culture solution when the seedlings were first transferred to the jars. In series, B this addition was not made until the seedlings had grown in the check solution for five weeks. In every other respect the corresponding cultures of the two series were treated alike.

Eighteen jars, numbered 1 to 18, were used. The five different solutions to be described presently were numbered 1 to 5 corresponding to contents of sodium chloride equivalent to osmotic concentration values of 0.0, 0.1, 0.2, 0.3, and 0.4 atmospheres, respectively. Jars 1 and 2 served as checks and received solution 1; jars 3 to 10 made up series A; jars 11 to 18 made up series B.

Solution 1, used in the checks, was one of those used by Tottingham (13) and designated by him  $T_1R_1C_6$ . This solution was chosen for the check, since it was found by Jones and Shive (6) to give good growth with soy beans, the only leguminous plant so far which has been given a systematic study with respect to its salt requirement. The total concentration, in terms of osmotic pressure, was one atmosphere, made up of the partial concentrations in atmospheres of the four salts used in this solution as follows: potassium nitrate, 0.1; monopotassium phosphate, 0.1; calcium nitrate, 0.5; magnesium sulfate, 0.3 atmospheres. The corresponding volume-molecular concentrations are: 0.0020, 0.0021, 0.0073, and 0.007.

The other four solutions, designated 2, 3, 4, and 5 were made up by adding sodium chloride, in increments of 0.1 atmosphere, until the maximum of 0.4 atmospheres was reached, at the same time maintaining approximately the original total osmotic concentration by elim-

inating correspondingly equivalent amounts of one or more of the other salts. For the first 0.1 atmosphere of sodium chloride introduced, the 0.1 atmosphere of potassium nitrate of the original solution  $T_1R_1C_5$  was eliminated, since the ingredients making up this salt are already present in some of the other salts (mono-potassium phosphate and calcium nitrate). In solutions 3 and 4, the further additions of sodium chloride replaced an equivalent and evenly distributed partial pressure of the other three salts. With solution 5, which contained 0.4 atmosphere of sodium chloride, the mono-potassium phosphate was restored to its original 0.1 atmosphere so as not to deprive the solution of two essential elements, the other two salts each being diminished by 0.1 atmosphere, thus bringing up the total concentration of this solution to 1.1 atmospheres.

The mere diminution in essential plant nutrients caused by eliminating part of the other salts should not be considered as in itself a factor of possibly grave consequences to plant growth, since previous studies with culture solutions by Trelease (15, p. 225) and others by Stiles (12) have brought out the fact that nutrient solutions within the range here used may vary considerably in either direction without showing any significant difference in their effect upon yield.

Sodium chloride of high purity was dried at 102°C. to constant weight, and a stock solution of 0.5 *M* concentration prepared. The formulae for the final dilutions were calculated and the culture solutions were finally prepared from the 0.5 *M* stock solutions. Iron in the form of a solution of ferrous sulfate was added at the rate of 0.1 mgm. of iron per liter of solution at the beginning; this amount was increased to 1 mgm. later.

The plants were grown in the greenhouse from January 3 to April 10, 1923, 98 days in all. During the last few weeks, half-gallon jars replaced the quart jars in which the plants were grown from the beginning. The solutions were renewed every three and one-half days.

Colorimetric determinations of the pH value of the discarded solutions, and occasionally of the original ones, were made according to the method of Gillespie (3), by means of the apparatus devised by Van Alstine (17), at various times during the growing period.

In the harvesting, drying, and weighing, the methods laid down in "A Plan for Co-operative Research" (8) were followed, omitting, however, the weighing of the green plants.

Determinations of total nitrogen and of chlorine were made separately of the tops and of the roots of the plants from each jar, following the analytical methods of the Association of Official Agricultural Chemists (1).

## DISCUSSION OF RESULTS

### *Yield in dry weight*

From table 1 and figure 1 it is seen that of the five solutions tested, solution 2, which is Tottingham's solution  $T_1R_1C_5$  modified by replacing the 0.1 atmosphere of potassium nitrate with an equivalent amount of sodium chloride, gave the highest yield in tops, roots, and consequently of the whole plant. This relation holds good for series A where the sodium chloride was introduced into the culture solution from the beginning, as well as for series B where the sodium chloride was not supplied until the plants had grown in the check solutions for five weeks. The greatest increase over the check manifested itself in the roots, amounting to 147 and 169 for series A and B, respectively, as compared with the check taken as 100. The values for the whole plant for each of the two series A and B are in fairly close agreement being 15.064 gm. and 15.476 gm., respectively, for the actual weights; and 124.8 and 128.2, respectively, for the relative weights. All the other cul-

TABLE 1  
*Effect of sodium chloride on yield, nitrogen and chlorine content of alfalfa grown in solution cultures (averages of duplicate cultures). Series A, sodium chloride used throughout the growth period, series B, sodium chloride introduced after plants had grown 5 weeks in the check solution*

	SOLUTION 1		SOLUTION 2		SOLUTION 3		SOLUTION 4		SOLUTION 5	
	Tottingham's TKIC <sub>4</sub>		0.1 atm. NaCl substituted for 0.1 atm. KNO <sub>3</sub>		0.2 atm. NaCl substituted for 0.1 atm. KNO <sub>3</sub> and 0.033 atm. each of the remaining salts		0.3 atm. NaCl substituted for 0.1 atm. KNO <sub>3</sub> and 0.066 atm. each of the remaining salts		0.4 atm. NaCl, 0.3 atm. substituted for 0.1 atm. each of KNO <sub>3</sub> , Ca(NO <sub>3</sub> ) <sub>2</sub> and MgSO <sub>4</sub> , and 0.1 atm. superimposed	
	Check series		Series A	Series B	Series A	Series B	Series A	Series B	Series A	Series B
Dry weight, grams	8.853	Tops.....	10.313	10.024	7.471	10.047	8.952	9.511	9.188	9.760
	3.214	Roots.....	4.751	5.452	3.131	5.100	4.160	4.610	4.645	4.329
	12.067	Whole plants.....	15.064	15.476	10.602	15.146	13.112	14.121	13.833	14.089
Relative dry weights	100	Tops.....	116.5	113.2	84.4	113.5	101.1	107.4	103.8	110.5
	100	Roots.....	147.8	169.6	97.3	158.6	129.4	143.4	144.5	134.7
	100	Whole plants.....	124.8	128.2	87.8	125.5	108.6	117.0	114.6	116.7
Nitrogen content, per cent	4.02	Tops.....	3.89	3.63	3.49	3.49	3.39	3.55	3.50	3.58
	2.40	Roots.....	2.60	2.29	2.30	2.37	2.18	2.27	2.20	2.49
	3.58	Whole plants.....	3.40	3.16	3.20	3.12	3.01	3.12	3.10	3.24
Chlorine content, per cent	Trace	Tops.....	0.23	0.32	0.50	0.49	0.63	0.68	0.77	0.78
	Trace	Roots.....	0.15	0.12	0.16	0.18	0.20	0.30	0.43	0.49
	Trace	Whole plants.....	0.21	0.25	0.40	0.39	0.50	0.56	0.65	0.69

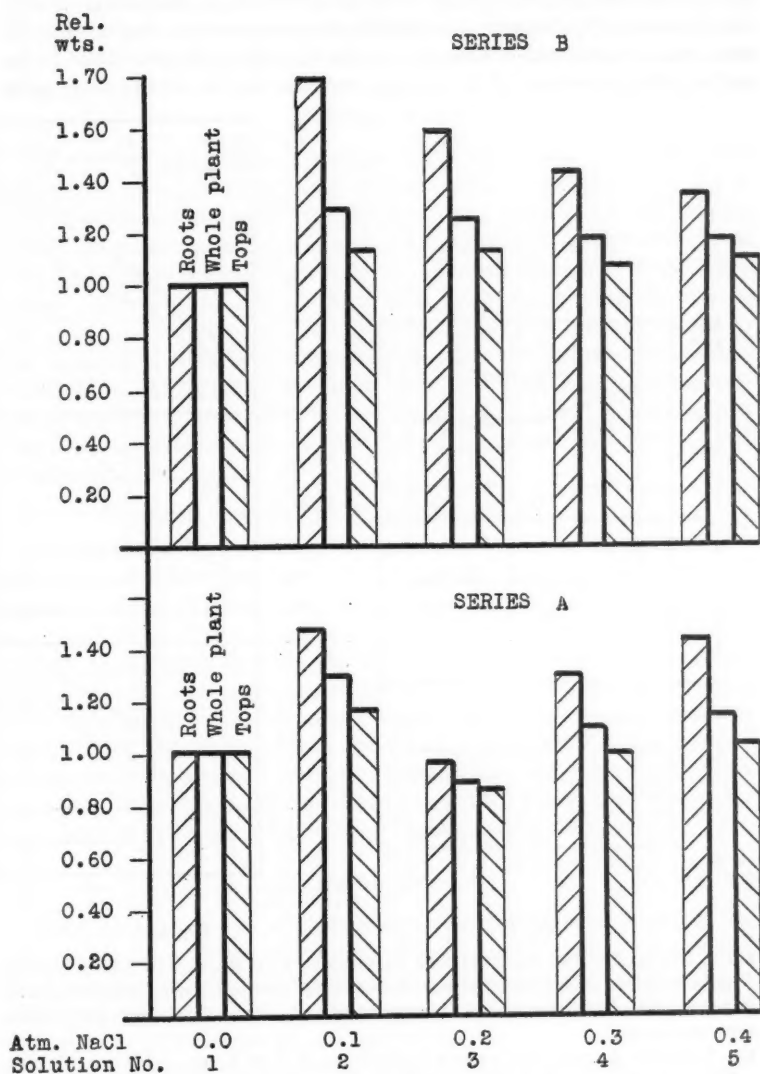


FIG. 1. GRAPHS SHOWING YIELDS OF TOPS, ROOTS, AND WHOLE PLANTS, RELATIVE TO CHECK (TOTTINGHAM'S SOLUTION  $T_1R_1C_4$ ) TAKEN AS 100

Series A, sodium chloride supplied from the beginning. Series B, sodium chloride supplied only after plants had grown five weeks in check cultures.

tures containing sodium chloride, with the exception of one culture of solution 3 in series A, also gave considerable increases over the check. The decrease noted in the case of solution 3, series A, is obviously not related to the sodium chloride content of the culture, since the parallel culture of the same

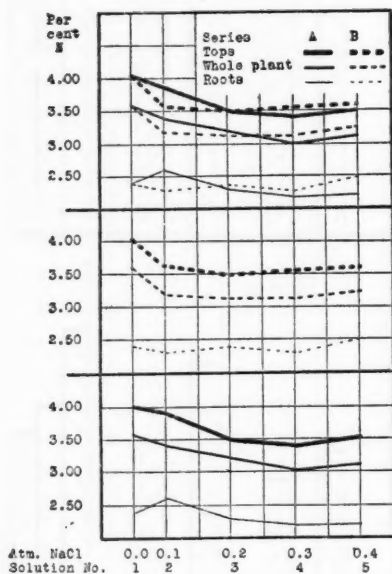


FIG. 2.

FIG. 2. GRAPHS SHOWING PERCENTAGE OF NITROGEN IN TOPS, ROOTS, AND WHOLE PLANTS Series A, sodium chloride supplied from the beginning. Series B, sodium chloride supplied only after plants had grown five weeks in check cultures. First point on each graph represents value of check.

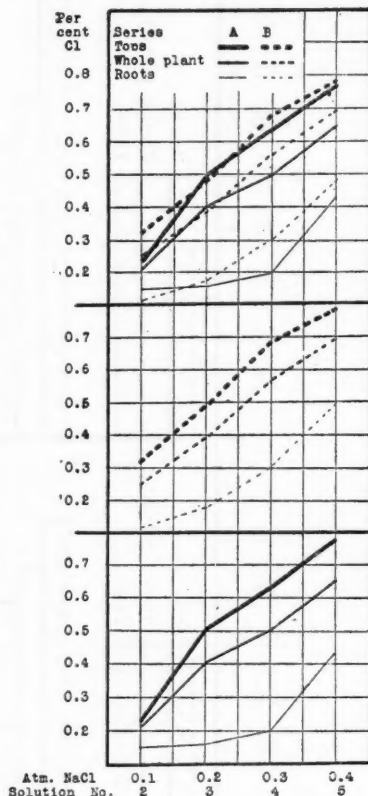


FIG. 3.

FIG. 3. GRAPHS SHOWING PERCENTAGE OF CHLORINE IN TOPS, ROOTS, AND WHOLE PLANTS Series A, sodium chloride supplied from the beginning. Series B, sodium chloride supplied only after plants had grown five weeks in check cultures.

solution in series B gave an increase over the check, practically equalling the highest obtained, and solutions with higher concentrations of sodium chloride also gave increases in yield, as already pointed out.



It is therefore allowable to conclude, within the limits of this preliminary study, that the inclusion of a certain proportion of sodium chloride among the salts used for solution cultures will, in the case of alfalfa, affect the yield advantageously.

#### *Nitrogen content*

Data for the percentages of nitrogen in the tops, roots, and whole plant, on the dry basis, are also given in table 1. While some fluctuation will be noticed when only the tops of the plants grown in different cultures are compared, this is less noticeable when a similar comparison is applied to the roots, and there are only small differences in the nitrogen content of the whole plants. This is brought out in the graphs (fig. 2) representing the data for the percentage of nitrogen. The diminished supply of nitrogen caused by the elimination of the 0.1 atmosphere of potassium nitrate and the final reduction of calcium nitrate to four-fifths of the original amount did not appreciably affect the nitrogen content of the plant as a whole. It is evident also that of the total nitrogen in the plant, by far the greater part was contained in the tops.

#### *Chlorine content*

The amount of chlorine taken up by the plants in the different solutions increased uniformly with the amount supplied. This, as seen in table 1, applies to the tops as well as to the roots and to series A as well as to series B. The graphs representing the chlorine content are practically straight lines for the tops in both series, the two lines almost coinciding. The graphs representing the chlorine content of the roots also run close together for both series, lying rather flat in the concentrations of 0.1 to 0.3 atmosphere osmotic concentration of sodium chloride, and taking a steep pitch in the solution containing 0.4 atmosphere, but never reaching the high points attained by the tops. There is no appreciable difference in the chlorine content of either roots, tops, or whole plants of the parallel cultures of the two series.

#### *Transpiration*

While no quantitative transpiration measurements were made on the different solutions, the greater amount of water lost in solution 2, containing 0.1 atmosphere of sodium chloride and which produced the highest yield in both series, was too obvious to escape notice. This difference can be noted in a rough way by comparing the quantity of liquid left in jars 3 and 11 (Plate 1), which represent cultures of solution 2 in series A and B respectively, with any of the others.

#### *Hydrogen-ion concentration*

The figures obtained for the pH values at various intervals of growth confirm the observation made by a number of investigators, that culture

solutions of this type in contact with the roots of growing plants tend to approach the neutral point with the advance of the growing period. The initial pH value of the solutions was 4.7 except in the case of solution 4, with the smallest amount of mono-potassium phosphate, which showed a pH value of 5.0. The discarded solutions showed an increasing pH value as the growing period advanced, reaching a pH value of 6.0 in a  $3\frac{1}{2}$ -day interval after the plants had been growing for three months. It is interesting to note that solution 4, which showed an initial pH value 0.3 higher than that of the other solutions, also showed a corresponding rise in the final pH value, which was 6.3 instead of the 6.0 of the other solutions.

#### SUMMARY AND CONCLUSIONS

1. Alfalfa was grown for 98 days in various culture solutions containing different amounts of sodium chloride, using as check the culture solution known as Tottingham's solution  $T_1R_1C_5$ .
2. The solutions containing sodium chloride gave higher yields than did the check.
3. The greatest increase was obtained with the solution in which 0.1 atmosphere osmotic concentration of sodium chloride was substituted for the 0.1 atmosphere of potassium nitrate of Tottingham's solution  $T_1R_1C_5$ .
4. The roots showed a greater relative increase than did the tops.
5. There was no appreciable difference in the nitrogen content of the whole plant, between the plants of the different cultures.
6. The chlorine content of the tops as well as of the roots increased uniformly with the increasing supply of that element, the tops, however, containing a larger percentage than the roots.
7. The greater part of both the nitrogen and the chlorine of the plant was contained in the tops.
8. The pH values of the discarded solutions approached gradually towards neutrality with the advancing growth of the plant.
9. Transpiration was highest in the case of the solution producing the largest yield.

#### ACKNOWLEDGEMENT

The ready assistance of Dr. John W. Shive of the Department of Plant Physiology, in the form of encouragement, suggestions as regards details of the work, and actual personal help at times, is hereby thankfully acknowledged.

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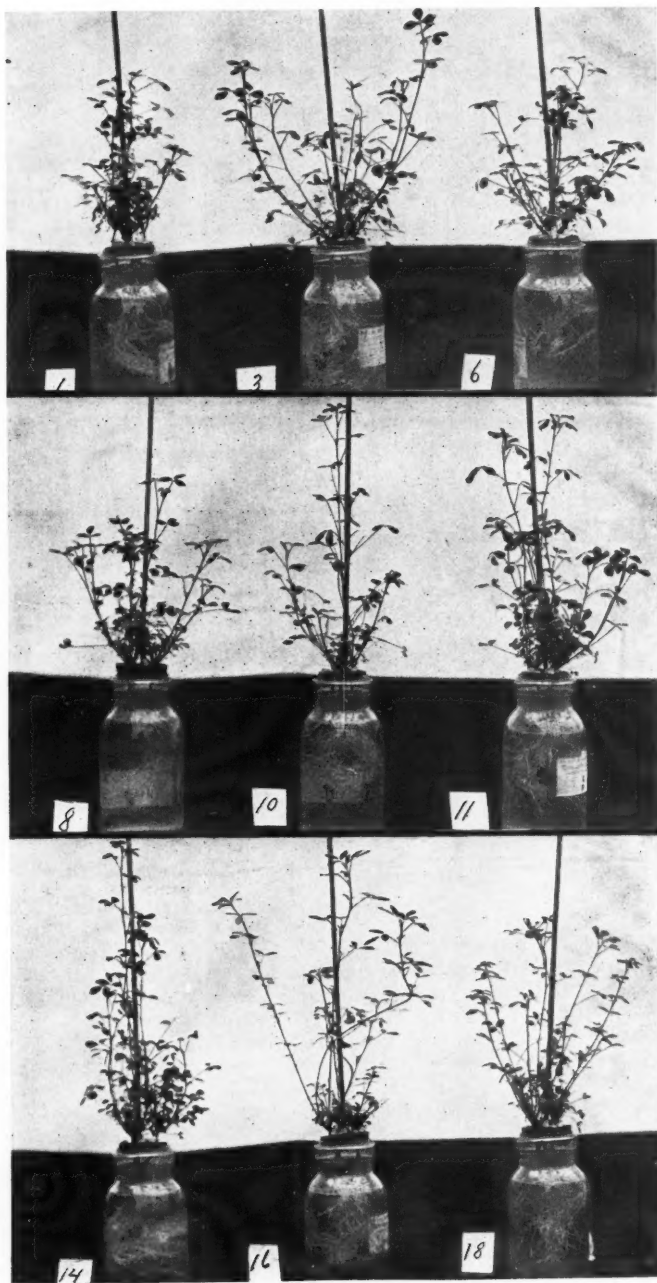
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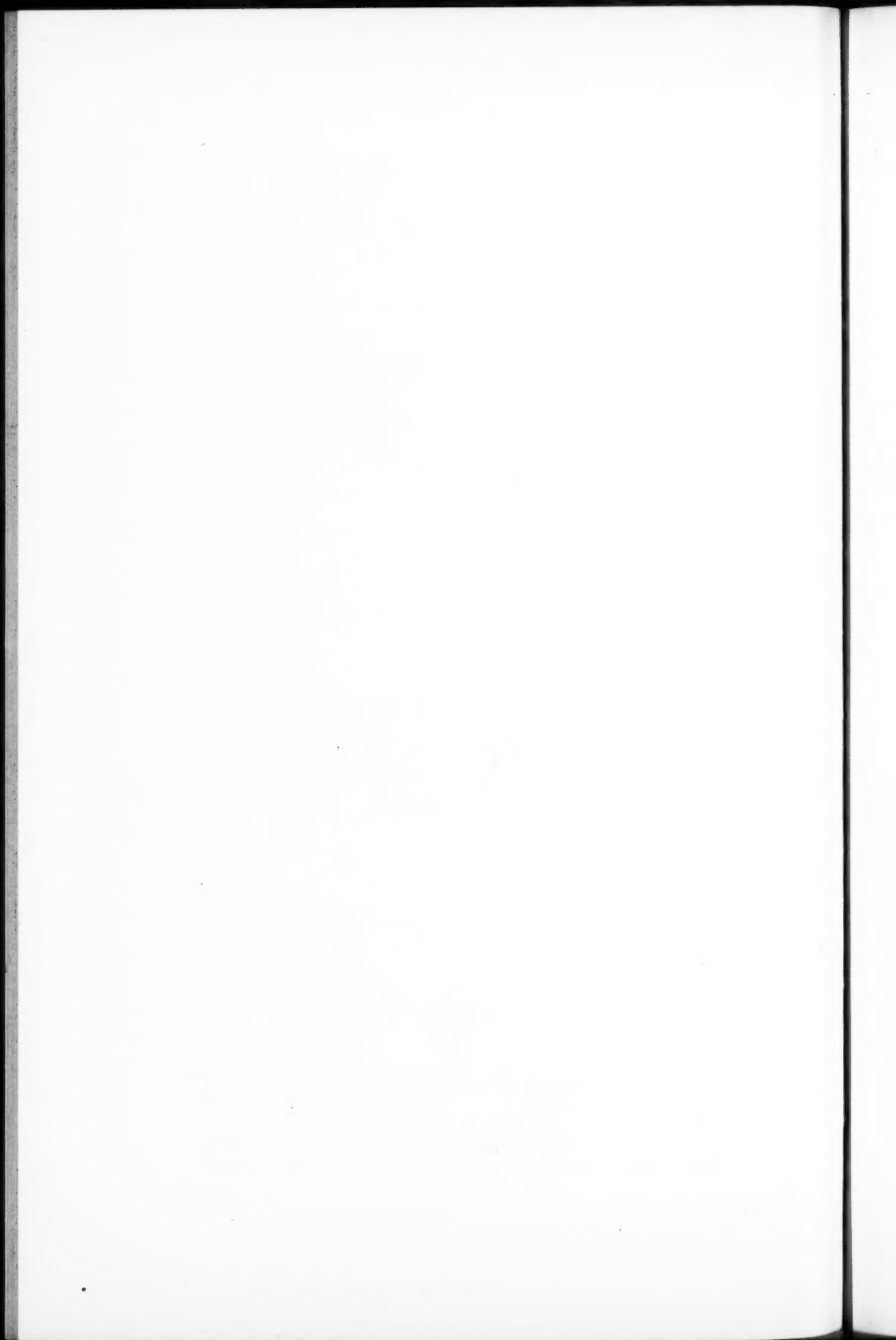
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## PLATE 1

## ALFALFA NINE WEEKS OLD, GROWN IN SOLUTION CULTURES

- Series A. Jar 1, check.  
Jar 3, 0.1 atm. sodium chloride.  
Jar 6, 0.2 atm. sodium chloride.  
Jar 8, 0.3 atm. sodium chloride.  
Jar 10, 0.4 atm. sodium chloride.
- Series B. Jar 11, 0.1 atm. sodium chloride.  
Jar 14, 0.2 atm. sodium chloride.  
Jar 16, 0.3 atm. sodium chloride.  
Jar 18, 0.4 atm. sodium chloride.







## ACIDITY OF HIGHLY BASIC SOILS

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Hawaiian soils while characteristically basic are with rare exceptions acid in reaction and usually show a high lime requirement by the Veitch method. On the other hand experiments and general observations have rarely indicated that sugar cane is benefited by liming these soils. As a whole the results indicate that sugar cane is indifferent to soil reaction or tolerates a certain kind of acidity. The question arises, what is the nature of this acidity?

Comparatively little work has been published showing the relation of lime requirement of soils as determined by the various methods to hydrogen-ion concentration in soils high in iron, aluminum and manganese oxides. In Hawaiian soils these oxides are often in excess of silica. Such a comparison has been recently made in this laboratory in order to obtain information relative to the nature of acidity in such types. To determine the merits of the different methods was not the purpose of this study.

### SOILS USED

Fourteen soils were selected varying in pH values from 4.63 to 8.01. The composition of these soils as to the more important basic constituents and silica is given in table 1. The results are expressed on the air-dry basis in order to illustrate the variation of the moisture content of the soil in the air-dry state.

Soil 848 is a heavy black clay adobe soil high in water-soluble iron and aluminum from windward Oahu, irrigated land and low elevation.

Soil 849 is the subsoil to no. 848 and similar in texture and color.

Soil 367 is a yellow clay loam. The analysis of soil 399 is not given in this table because of its similarity to no. 367. Both are from the island of Kauai, upland soils from unirrigated fields.

Soil 186 is a sandy loam high in organic matter and combined water from Honokaa, island of Hawaii.

Soil 187 is the subsoil to no. 186 and similar in texture but lighter in color.

Soil 765 is a yellow silty loam from the Hilo-Hamakua district. Rainfall in this district is very heavy, land unirrigated, clay highly hydrated and high in organic matter.

Soils 621 and 408 are red clay loams from districts where rainfall is low. The former is from Lahaina, Maui, medium elevation and the latter from low elevation on Oahu, Ewa plantation.

Soil 409 is the subsoil to no. 408.

Soil 734 is a chocolate colored silt loam from a manganiferous area, an upland soil on the island of Oahu.

Soil 722 is a brown clay loam, island of Oahu, lowland irrigated land.

Soil 872 is the same type as no. 848 except taken from a section nearer the sea and high in coral.

Soil 3 is a sandy clay loam from Honolulu derived from volcanic ash and underlaid with it. While it is high in lime there is practically none present as carbonate.

These soils have been selected from sugar lands located on all the four major island, Oahu, Maui, Kauai and Hawaii and represent lowlands, uplands, humid and more or less arid districts.

TABLE 1  
*Partial analyses of soils\**

SOIL NUMBER	TiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	Mn <sub>2</sub> O <sub>4</sub>	CaO	SiO <sub>2</sub>	LOSS ON IGNITION	
							H <sub>2</sub> O	Organic matter, etc.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
848	6.0	11.17	19.33	0.18	1.23	40.9	12.47	10.63
849	7.2	10.37	18.88	0.13	1.42	39.8	14.20	9.11
367	12.2	22.54	28.51	0.20	0.61	9.8	9.06	15.80
765	8.0	22.54	15.46	0.25	1.15	15.1	12.03	11.57
186	6.0	13.56	15.09	0.26	1.23	13.2	19.60	29.63
187	4.4	13.56	18.04	0.25	1.01	17.5	22.20	29.63
621	7.4	21.74	27.36	0.30	0.98	31.7	4.11	10.69
409	10.0	15.56	25.64	0.22	0.84	33.8	9.10	9.09
734	5.8	14.16	28.79	1.10	0.92	33.9	8.90	11.25
722	9.8	15.76	28.24	0.43	0.86	31.0	6.04	13.34
408	9.4	14.56	26.04	0.77	0.99	33.7	7.70	11.5
872	5.6	13.96	15.44	0.34	3.14	42.3	9.17	10.58
3	5.9	23.74	15.51	0.40	3.25	30.8	7.00	10.24

\* Analyses by fusion with sodium carbonate.

#### METHODS USED FOR DETERMINING LIME REQUIREMENT

The lime requirement or acidity of a soil is usually attributed to such phenomena as the actual presence of mineral or organic acids, absorptive or adsorptive properties of colloidal material, free hydrogen ions or the presence of certain specific compounds which on hydrolysis or replacement increase the hydrogen-ion concentration of the soil solution.

Extensive reviews of the various methods are available (1) which makes such a review unnecessary here. Those used in this investigation include the treatment of the soil with the salts of strong acids and strong bases, for example the Hopkins method (2) which calls for the use of KNO<sub>3</sub> or KCl; weak acids and strong bases, for example the Jones method (3) using a solution of calcium acetate, the Loew (10) method using potassium acetate and the Carr (5) method using KSCN; weak acids and weak bases for example the Truog (12) method using zinc sulfide. Other methods include the Veitch (13) and the Hutchinson and MacLennan (9, p. 75) methods which utilize solutions of Ca(OH)<sub>2</sub> and CaH<sub>2</sub>(CO)<sub>3</sub> respectively, the Lyon and Bizzell method (4) in which Ba(OH)<sub>2</sub> is used in a somewhat similar manner, the Conner method (6) and that of Rice and Osugi (11) in which the hydrolysis of esters and sucrose respectively are used, Hollemann's method (9, p. 81) which measures the percentage

of lime soluble in water saturated with  $\text{CO}_2$ , and Immendorf's method (9, p. 82) in which a back titration of 0.2 *N*  $\text{H}_2\text{SO}_4$  in which the soil has been boiled is used.

A number of qualitative tests were also applied to these soils which include the litmus test, those of Veitch and Truog already described, the Comber method (7) which uses an alcoholic solution of KSCN the Loew (10) method which uses a solution of KI in starch paste and as modified by Daikuhara using starch iodide paper instead of starch paste as a test for free iodine.

#### QUALITATIVE METHODS

The results obtained by the qualitative methods are given in table 2.

Of the above qualitative methods the litmus, Veitch and Comber appear to be best suited to local soil types as an indication of absence of adequate lime supply. The Loew and Truog methods appear to be of little value taking the pH values as a standard for comparison. The reaction involved in the two latter methods requires the presence of an acid which in the Loew and Loew-

TABLE 2  
*Qualitative tests for acidity*

SOIL NUMBER	REACTION BY VARIOUS METHODS						pH VALUES
	Litmus	Veitch	Loew	Loew Daik.	Truog	Comber	
848	Acid	Colorless	Blue	Colorless	Positive	Dark red	4.63
849	Acid	Colorless	Blue	Colorless	Positive	Dark red	4.80
367	Acid	Colorless	Colorless	Colorless	Positive	Faint red	4.88
399	Acid	Colorless	Colorless	Colorless	Negative	Faint red	4.97
765	Acid	Colorless	Colorless	Colorless	Negative	Faint red	5.56
186	(?)	Colorless	Colorless	Colorless	Negative	Faint red	5.73
187	(?)	Colorless	Colorless	Colorless	Negative	Faint red	5.98
621	(?)	Colorless	Blue	Colorless	Negative	Faint red	5.98
409	(?)	Colorless	Colorless	Colorless	Negative	Faint red	6.32
734	(?)	Colorless	Colorless	Colorless	Negative	Colorless	6.66
722	Neutral	Colorless	Colorless	Colorless	Negative	Colorless	7.00
408	Neutral	Colorless	Colorless	Colorless	Negative	Colorless	7.08
872	Alkaline	Red	Colorless	Colorless	Negative	Colorless	7.67
3	Alkaline	Red	Colorless	Colorless	Negative	Colorless	8.01

Daikuhara methods sets free iodine from KI as indicated by the starch paste of starch iodide paper and which in the Truog method liberates  $\text{H}_2\text{S}$  from  $\text{ZnS}$  as indicated by the lead acetate test paper. These methods apply only in those soils with pH values of 4.8 or less and indicate that these reactions are inhibited in our soil types or that in those soils of low hydrogen-ion concentration the small amounts of iodine or  $\text{H}_2\text{S}$  are absorbed and not set free on boiling. The Comber method showed some promise of yielding information relative to the nature of acidity and in view of this fact and the more advanced conceptions of soil acidity in which the salts of iron and aluminum play an important part more time was devoted to a study of this method. The reagent used in this test is an alcoholic solution of KSCN which will, in the presence of soluble iron, increase the concentration of  $\text{Fe}(\text{SCN})_3$  in the liquid phase to

such an extent as to greatly increase the delicacy of the reaction. From the composition of Hawaiian soils one would ordinarily attribute acidity to be in a large part due to these elements.

According to Comber the red color increases in depth on standing. As thus applied to Hawaiian soils  $MnO_2$  which is present in varying amounts in practically all types introduces a factor which within a certain pH range materially enhances the value of this method or viewed in another light may add to its value. In table 3 are noted the observations made with this test immediately after the soil had settled and at the close of 24- and 48-hour periods.

TABLE 3  
*Showing the factor of time in the Comber method*

SOIL NUMBER	pH VALUES	COLOR REACTION AT CLOSE OF VARIOUS PERIODS OF TIME		
		15 min.	24 hr.	48 hr.
848	4.63	Dark red	Dark red	Dark red
849	4.80	Dark red	Dark red	Dark red
367	4.88	Light red	Light red	Light red
399	4.97	Red	Red	Red
765	5.56	Red	Red	Red
186	5.73	Light red	Light red	Light red
187	5.98	Light red	Light red	Light red
621	5.98	Light red	Blue	Blue
409	6.32	Light red	Blue	Blue
734	6.66	Colorless	Blue	Blue
722	7.00	Colorless	Blue	Blue
408	7.08	Colorless	Blue	Blue
872	7.67	Colorless	Colorless	Blue
3	8.01	Colorless	Colorless	Colorless

This blue color was found to be due to  $MnO_2$  and it is apparent from table 3 that we may expect soils of pH less than 5.5 to show a permanent test for soluble iron. Within the range of pH 5.5-7.0,  $MnO_2$  will change the red color to a greenish blue. This change in color is due directly to the  $MnO_2$  and not to soluble manganese salts. For example, on adding  $MnO_2$  to 848 there resulted a gradual fading to a blue while on adding manganese salts there was no change in color, the red color of undissociated  $Fe(SCN)_3$  being permanent.

It was further suggested by Comber that if  $FeCl_3$  be added to this reagent thus developing the red color before shaking with the soil, the iron present in the liquid phase will be displaced by lime in alkaline soils. As thus applied to the soils under investigation further peculiarities were met. The observations noted were as follows. In soils 848 to 187 there was no greater amount of color than where no  $FeCl_3$  was added. Soils 621 to 408 all showed no greater amount of color and turned blue in 24 hours. While 872 and 3 remained colorless. The results clearly show the high absorptive capacity of the soil colloids and the influence of hydrogen-ion concentration on the color of the

$\text{Fe}(\text{SCN})_3$ . The delicacy of the reaction in these soils is greatly increased by using an ether-alcohol reagent.

TABLE 4  
*Comparison of pH values with lime requirement in pounds  $\text{CaCO}_3$  per acre*

SOIL NUMBER	pH VALUES	LIME REQUIREMENT BY VARIOUS METHODS						
		Veitch	H-MacL	Lyon-Biz.	Hopkins	Loew	Jones	Carr
		lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
848	4.63	11460	11080	11480	3570	9360	6840	7020
849	4.80	11460	13440	12820	6880	11580	8570	9000
367	4.88	6390	9070	8060	170	3630	4860	1200
399	4.97	6750	6720	7990	100	3510	3600	
765	5.56	15120	11500	12300	100	4410	6480	4290
186	5.73	22500	9820	13230	170	6480	6480	2200
187	5.98	24510	9570	11380	170	2490	5580	1000
621	5.98	2340	1510	250	85	1480	1260	
409	6.32	1350	2680	200	60	810	900	
734	6.66	1350	3520	120	40	1080	1260	
722	7.00	660	126	60	40	420	360	
408	7.08	1680	126	310	40	330	360	
872	7.67	660	-2680	Alk.	Alk.	270	Alk.	
3	8.01	660	-6630	Alk.	Alk.	210	Alk.	

TABLE 5  
*Comparison of pH values with lime requirement determinations*

SOIL NUMBER	LIME REQUIREMENT BY VARIOUS METHODS			
	Rice Osugi	Conner	Immendorf	Holleman
	gms. $\text{CuO}$ per 2.5 gm. soil	cc. 0.5 N alkali per 10 gm. soil	cc. 0.2 N acid neutralized by 10 gm. soil	per cent $\text{CaO}$ soluble in $\text{CO}_2$ -saturated water (1 part soil to 5 parts water)
848	0.0750	9.6	13.25	0.008
849	0.1160	4.4	13.40	0.007
367	0.0190	13.0	8.50	0.009
399	0.0130	10.0	7.75	0.013
765	0.0536	24.0	15.90	0.015
186	0.0254	10.0	20.25	0.017
187	0.0520	6.0	20.10	0.016
621	0.0150	8.0	10.00	0.023
409	0.0025	2.8	11.75	0.020
734	0.0050	10.0	14.90	0.023
722	0.0153	2.0	15.10	0.033
408	0.0136	2.8	13.00	0.027
872	0.0395	4.4	21.10	0.081
3	0.0230	2.0	24.10	0.131

#### QUANTITATIVE METHODS

A comparison of the quantitative methods of determining acidity or lime requirement with pH values is given in tables 4 and 5. Table 4 includes those

methods which express lime requirement as pounds of  $\text{CaCO}_3$  per acre and table 5 includes those in which other terms are used.

Let us first consider the results obtained with the Veitch, Hutchinson-MacLennan and Lyon-Bizzell methods which, in a manner, involve similar principles. In the two former methods, amelioration of soil acidity is effected by methods closely resembling field practice, a solution of  $\text{Ca}(\text{OH})_2$  in the one case and  $\text{CaH}_2(\text{CO}_3)_2$  in the other. It is evident, however, that in addition to a neutralization of actual acidity and a replacement of soluble iron, manganese and aluminum the acidity will appear greater than it really is because of the physical absorption by the colloids. This extra lime, however, may be of benefit to the soil in other ways than neutralization of soil acidity.

In the results obtained by these three methods there is a general agreement in the soils with pH values of 4.6-5.5. Soil 186 and subsoil 187 show a wide variation. It is evident that this type of acidity is due to several acid constituents as shown by the different degrees of reaction with the different basic combinations. Absorption as a factor in this soil might be interpreted from the high content of water of hydration and the resulting colloidal properties usually associated with such types. Comparing soils 187 and 621 it will be noted that both show a pH of 5.98 while there is a difference of 10 tons lime requirement by the Veitch method, 4 tons by the Hutchinson-MacLennan method, and 5.5 tons by the Lyon-Bizzell method. These results indicate a high potential acidity in the former probably due to the presence of acid-reacting organic compounds and an acidity of greater intensity in the latter soil which has a lower organic content. The soils of lower hydrogen-ion concentration given in the table all show a greater degree of variation. In general it may be said that these methods neutralize all types of acidity and will usually agree closely enough for practical purposes on all our highly acid types where the ratio of lime absorbed by the colloids to that used in neutralizing acidity is low. But in those less important soils with pH values of 6-7 in which there is a higher ratio of absorbed lime to that neutralizing the soil acids, there is greater variation and less agreement, the Veitch method showing absorption even in the alkaline soils. Specific information relative to the nature of the acidity is wanting except that the Veitch method gives higher results (compared to the actual hydrogen-ion concentration on soils the acidity of which is due to organic matter) than where due to factors other than organic matter. In other words, the Veitch method has the advantage in the case of soils of high potential acidity where it is desired to estimate the lime needed to neutralize the soil, since in such types this factor is not indicated by the hydrogen-ion concentration.

In the Hopkins, Jones, and Loew methods we have the much discussed acidity by replacement or absorption. It is generally conceded that these methods indicate mineral acidity which is shown by the presence of aluminum, iron and manganese in the extracts of soils treated with solutions of the salts which are the basis of the above methods and supported by the fact that the



salts of these elements react acid through hydrolysis. Practically all soils show a higher acidity where acetates are used than with chlorides or nitrates and this is strikingly true of Hawaiian soils. Even in our most acid types it will be noted that the lime requirement as indicated by the Hopkins method is practically negligible. Both KCl and  $\text{KNO}_3$  were used and the results obtained with both salts checked very closely. It is significant that the highest results were obtained on the soils of highest hydrogen-ion concentration and in which soluble iron and aluminum salts were present in large amounts, and that in these soils potassium acetate gave higher results than the calcium acetate.

The Conner method and Rice-Osugi method show a wide variation in the hydrolytic action of these soils as measured by the hydrolysis of sucrose and ethyl acetate. These compounds are hydrolysed or decomposed in the alkaline soils as well as the acid and there appears to be little relation between the hydrogen-ion concentration and hydrolysis indicating that this reaction is due in part to other factors than acidity.

The Immendorf method which acts as a measure of the soluble bases is of little or no value in these highly basic soils either as a measure of lime requirement or as indicating the nature of acidity. All the acid extracts of these soils were high in iron and aluminum except for soil 3.

On the other hand the Hollemann method which measures the solubility of lime in water saturated with carbon dioxide shows a very close relation to pH values. There is a gradual increase in the solubility of lime in this reagent with decrease in hydrogen-ion concentration. These results clearly indicate that the solubility of lime is definitely associated with the hydrogen-ion concentration of these highly basic soils both as a neutralizer of actual soil acidity and in the replacement of iron, aluminum and manganese in the soil solution because of their relative positions in the electromotive series. The results obtained show beyond a doubt that acidity in Hawaiian soils is largely a question or presence of absence of readily available calcium compounds.

Carr (5) has developed a quantitative method from that of Comber using the red color of  $\text{Fe}(\text{SCN})_3$  as an indicator after determining the pH value at which this salt becomes colorless. As thus applied to Hawaiian soils some interesting results were obtained. In highly acid soils which give a positive test for iron the titration is to a greenish blue rather than a colorless solution. A pH value of 5.4 is the turning point and the change is rather indefinite. However in titrating from colorless or blue to red the change is even yet more indefinite and in the highly manganiferous soils considerable acid may be added without changing the pH value. For example, an acid-reacting soil containing 7 per cent  $\text{MnO}_2$ , having a pH value of 5.9, on titrating with 0.1 *N* alcoholic  $\text{H}_2\text{SO}_4$  still showed a pH value of 5.6 after adding 120 cc. and the red color of  $\text{Fe}(\text{SCN})_3$  had not yet appeared. These observations are of considerable value in interpreting the rôle of manganese in the acidity of our manganiferous soils. Where appreciable amounts of manganese are present

as dioxide this compound will tend to stabilize the soil reaction and maintain a certain definite range of hydrogen-ion concentration.

#### DISCUSSION

In attempting an interpretation of this data as a whole, the results show that the acid soils of the humid districts will contain considerable acid-reacting organic matter of high potential acidity not indicated in the hydrogen-ion concentration. Such types will usually show a very high lime requirement but not necessarily a low pH value. Other factors may be absorption or adsorption, presence of silicic acid, complex acid-reacting silicates and hydrolyzable salts of aluminum, iron and manganese.

TABLE 6  
*Fe<sub>2</sub>O<sub>3</sub> and Al<sub>2</sub>O<sub>3</sub> in the salt extracts of acid soils*

SOIL NUMBER	pH VALUES	IN KNO <sub>3</sub> EXTRACT	IN KC <sub>2</sub> H <sub>3</sub> O <sub>2</sub> EXTRACT
		<i>per cent</i>	<i>per cent</i>
848	4.63	0.228	0.183
849	4.80	0.220	0.192
367	4.88	0.043	0.017
399	4.97	0.044	0.024
765	5.56	0.082	0.027
186	5.73	0.085	0.056
187	5.98	0.073	0.022
621	5.98	0.100	0.086
409	6.32	0.189	0.180
734	6.66	0.072	0.097
722	7.00	0.113	0.158
408	7.08	0.194	0.196
872	7.67	0.268	0.252
3	8.01	0.126	0.174

In ascertaining the rôle of these elements, their presence is usually sought in the salt solution extracts. Table 6 gives the percentage of iron and aluminum oxides in the potassium nitrate or Hopkins method extract and the potassium acetate method or Loew extract of these soils.

It has usually been noted that (6) the aluminum content of the acetate extract is lower than that of the nitrate. This is explained theoretically by the difference in the hydrolytic products of the reactions involved, namely, aluminum acetate of which the products of hydrolysis are aluminum hydrate and free acetic acid and aluminum nitrate which is retained in solution as the acid salt. In the acid soils with reactions of pH 4.6-6.3, the iron and aluminum content is less in the acetate than in the nitrate extract. While in the soils with reactions of pH 6.6-8.0 this relation does not hold.

Judging from the color of the ammonia precipitate and qualitative tests, iron was present principally in the extracts of the most acid soils while scarcely more than a trace was found in the extracts of the soils with pH values of 6.0

to 8.0. It should be noted that the extracts of the alkaline soils contained just as much or possibly more iron and aluminum than those from the acid soils. The formation of acid salts is apparently inhibited by the association with higher soluble lime content.

This influence of easily soluble bases is apparently a very important factor. The solubility of silica and silicates is also of some importance. The ratio of iron, aluminum, calcium and silica was determined using 0.2 *N* nitric acid as a solvent and the results are given in table 7.

It will be noted that in all types easily soluble aluminum is far in excess of iron and highest in the highly organic soils. In general all four constituents show wide variations in the different soil types and there appears to be no

TABLE 7  
*Easily soluble bases and silica in 0.2 N nitric acid extract*

SOIL NUMBER	SiO <sub>2</sub>	Fe <sub>2</sub> O <sub>3</sub>	Al <sub>2</sub> O <sub>3</sub>	CaO	pH VALUES
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
848	0.120	0.032	0.468	0.435	4.63
849	0.137	0.026	0.052	0.396	4.80
367	0.012	0.005	0.313	0.051	4.88
399	0.012	0.100	0.182	0.108	4.97
765	0.097	0.022	1.518	0.145	5.56
186	0.252	0.043	3.920	0.423	5.73
187	0.386	0.032	3.830	0.141	5.98
621	0.067	0.008	0.175	0.176	5.98
409	0.105	0.007	0.138	0.202	6.32
734	0.270	0.004	0.537	0.278	6.66
722	0.116	0.006	0.233	0.253	7.00
408	0.141	0.009	0.174	0.269	7.08
872	0.287	0.031	0.602	1.045	7.67
3	0.493	0.011	1.585	1.185	8.01

relation between this ratio and acidity. Apparently the mineral acidity or hydrogen-ion concentration is not primarily entirely a function of the solubility of these elements but rather a function of other factors which limit the formation of acid or basic combinations.

Eliminating such abnormal types as soils 848 and 187 and considering only those in the series which are more typical of the average island soils, there is a tendency toward decreasing solubility of aluminum, less so the iron and an increase in soluble calcium with decrease in hydrogen-ion concentration. This ratio probably has an important bearing on the mineral acidity or acid reacting compounds of these elements.

#### EVIDENCE OF MINERAL ACIDITY OR PRESENCE OF ACID REACTING ORGANIC COMPOUNDS

As previously noted in the ammonia precipitates obtained on the nitrate and acetate extracts only traces of iron were found even in soils 848 and 849

both of which gave strong tests for soluble iron salts by the Comber method. This type of acidity is greatest in the heavy clay soils in which drainage is poor and aeration low and of which the above samples are typical. Iron then is not an important factor in the acidity of average island soils and its activity is confined to the most acid types and even in these evidently plays a secondary rôle to aluminum.

Manganese on the other hand is a factor only in those soils of low acidity. Of the manganiferous samples examined, the pH values were all 5.9 or higher. It was noted also that in an examination of the subsoils from these types that in all cases the subsoil was of a lower hydrogen-ion concentration than the top soil. Expressed in pH values the difference was 0.6 to 0.9 less acid. Judging from the relative positions of calcium and manganese in the electromotive series one would theoretically anticipate this to be true. The calcium apparently replaces the manganese in the soil solution which usually occurs on the soil grains as a coating, while the characteristically open texture of the manganiferous soils favors the leaching of lime into the subsoil.

Aluminum is present in easily soluble form in all the island types, highest in the humid districts and lowest in the arid. Apparently one of the principal rôles which lime plays in Hawaiian soils is in its relation to the acidity of aluminum salts which appear to be present in soluble form even in the alkaline types. This is best shown by the progressive increase in per cent of lime soluble in water saturated with  $\text{CO}_2$  with increase in pH values and the further fact that aluminum appears to be present in equally available form in the acid and alkaline soils. In other words, the aluminum may be present either as an acid or neutral salt depending upon environment. It is significant that soil 186 represents a comparatively unproductive area, much less productive for example, than soil 848 which is much heavier and very poorly aerated. It has been found, in our island soils, that phosphate is much more available in the lowland sections than in the uplands. The principal inherent difference in the soils from such districts is the higher acidity and lower lime content of the uplands and a greater rainfall. A study of this phenomenon indicates that in the absence of lime in the more acid upland soils, phosphoric acid has combined with aluminum. The resulting compound has become hydrated and hence less available as there is no consistent difference in the total phosphate content of upland and lowland soils. The only variation is in the availability. It is believed that these facts lend some indication of the presence of acid mineral salts and that they are a factor in the acid reaction of island types.

The importance of silicates as a factor in this acidity appears to be at least closely related to that of aluminum. Silicates are present in Hawaiian soils in a comparatively soluble form as shown by its presence in the waters of the island streams and its solubility in the dilute and strong acid extracts of the soil. It will be noted in table 4 that the lime requirement as determined by potassium acetate is greatly in excess of that shown by extraction with a solution of potassium nitrate. In the case of aluminum silicate, the acidity

from potassium acetate is due entirely to acetic acid formed from the hydrolysis of aluminum acetate. There results a greater acidity than where acid salts are formed and relatively less aluminum in solution.

When anhydrides become hydrated they tend to assume acid or basic properties. This would apply to oxides of iron and aluminum as well as silicates and it is evident, therefore, that these compounds are more or less amphoteric depending upon environment. In our humid districts or those in which rainfall is heaviest a rapid leaching of lime has been noted. Where present as a double silicate of calcium and aluminum there may result a complex aluminum silicate containing no lime and of more acid tendencies. Soluble forms of lime have been found to be very low in the soils from these districts. The acid or neutral state of aluminum-silicates will also depend upon the amount of water of hydration and ratio of silica to aluminum. Conner (6) found that ignition destroys the acid-reacting properties of clays as determined by the Hopkins method. Loss of water of combination therefore lowers the acidity of aluminum silicates. He found that those silicates of low water of combination were not acid and that those containing considerable water and silica were highly acid. Applying these theories to the island soils we find the soils from the districts of low rainfall to be low in acidity while the reverse is true of the soils from humid districts. Examples of the former are soils 621, 408, 409, 734, 722 while soils 765, 186, 187 will illustrate the latter. The higher organic content of the latter, however, must not be overlooked. The largest humid district under cane cultivation in Hawaii is the Hamakua coast section on the island of Hawaii. Available lime and potash are low in this section and water of combination or hydration high. The moisture content of the air dry soils is often as high as 25 per cent and the maximum water-holding capacity over 100 per cent. Such conditions actively favor the formation of acid aluminum silicates. There is a notable response to potash fertilization in this district indicating the absence of available forms of potash silicates. There is a progressive decrease in rainfall from the Hilo section of this coast to the Kohala district at the northern most extremity of the island. Accompanying this decrease in rainfall there is a decrease in maximum water-holding capacity, water of hydration and acidity of the soil. These observations tend to prove that the acidity of our humid districts is due in large part to aluminosilicic acids or acid silicates. In the more arid districts where cane is grown under irrigated conditions, rainfall being too low to support maximum growth, we find more lime present in soluble forms. In these districts there is decidedly less response to potash manuring. It may be of interest to state that the water of hydration is lower in the soils from these districts. We have under these conditions more active double silicates of potash, lime, soda or magnesia and aluminum which partially prevent the formation of aluminosilicic acids through neutralization.

## SUMMARY

In this paper are reported the results obtained by the Hopkins, Jones, Loew, Carr, Truog, Veitch, Hutchinson-MacLennan, Lyon-Bizzell, Conner, Rice-Osugi, Hollemann, Immendorf and hydrogen electrode methods of determining soil acidity as applied to soils high in oxides of iron and aluminum.

While some comments have been offered regarding the merits of these methods on such soil types the main purpose has been to interpret from the data the nature of the acidity in our island types of soil.

In the humid districts acid-reacting organic matter is an important cause of soil acidity while in those sections of low rainfall this is true to a far less extent.

Mineral acidity is due in most part to aluminum salts and aluminum silicates, the latter predominating in the humid districts. Iron is a factor only in the very acid soils with reactions of pH 4-6 while manganese is a factor only in those with pH values of 5.5 to 7.0. Water of combination or hydration is also an important factor of soil acidity in all types of soil studied.

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## THE OCCURRENCE AND ACTION OF FUNGI IN SOILS<sup>1</sup>

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Although soil fungi have been studied to some extent, our knowledge of the occurrence and functions of these organisms is still comparatively limited. In fact it is only recently that they have been looked upon as being of any importance in the soil. Many of the earlier workers considered them contaminations and believed their occurrence in the soil was accidental. Recent investigations have shown, however, that certain fungi may be more active than bacteria under some conditions, and that, although their functions as a whole are neither as varied nor as essential as those of many bacteria, they play an important rôle in soil fertility. Their wide distribution and large numbers, especially under conditions where bacterial growth is inhibited, indicate that they may take an active part in many soil processes.

That fungi exist in the soil in an active state is no longer open to question. Vegetative growth is often so abundant that the mycelium is plainly visible on the surface and within clumps of soil. This is especially true in the presence of large quantities of organic matter. But where the mycelium is not evident to the naked eye, its presence may be demonstrated with the method employed by Waksman (18). Furthermore, as Brown and Halversen (1) point out, "The presence of mold spores in a soil not only shows the previous occurrence of active forms, but what is more important, it shows the future growth of mycelial forms." Hence fungi undoubtedly have a potential influence, at least, on fertility.

Russell (15) has well stated the present situation regarding soil fungi when he says, "It is impossible on present knowledge to assess the importance of fungi in soil fertility. The decomposition of cellulose is undoubtedly important and justifies the old view that fungi are the humus formers of the soil. On the other hand the locking up of nitrogen compounds is a disadvantage, which, however, would be considerably counterbalanced if the substance of the mycelium were readily decomposable by bacteria on the death of the organism."

### OBJECT

It is evident, then, that further work is needed to supply the true facts concerning soil fungi and to permit of the determination of their relation to

<sup>1</sup> The author wishes to express his appreciation to Dr. P. E. Brown and to Dr. Paul Emerson for outlining the problem and for many helpful suggestions during the course of the work, and to Dr. J. C. Gilman for his assistance in identifying the species.

soil problems. The purpose of the present work was to study certain activities of various fungi in soils, especially in relation to sulfur and phosphorus, which have received little attention heretofore.

Definite information regarding the fungous flora of the soil is very desirable. Until it is known with reasonable certainty what forms occur in the soil and just what reactions each organism will bring about, the actual importance of fungi in relation to soil problems cannot be definitely determined. While time has not permitted a complete determination of the fungous flora, members of the common soil genera have been isolated and studied in this work.

#### TAXONOMIC STUDIES

Five soils from the agronomy farm at the Iowa State College were used in the isolation work. The lime requirement of these soils varied from zero to 5 tons per acre as measured by the Truog method. The soil types represented and the crops growing on the soils were as follows:

SOIL TYPE	LIME REQUIREMENT	CROP
Webster clay loam.....	0	Clover
Webster silty clay loam.....	1 ton	Corn
Carrington loam.....	2 tons	Clover
Carrington loam.....	3 tons	Clover
Carrington loam.....	4-5 tons	Clover

Samples were taken in the usual way to depths of 3 to 4 inches, sterile instruments being used to prevent contamination. The soils were taken to the laboratory, dilutions made and plates poured immediately. The media used were modified albumen agar, Cook's No. 2 agar, Czapek's synthetic agar, and Waksman and Fred's (20) synthetic acid agar. Plates were poured in triplicate on each medium.

As soon as the colonies on the plates began to sporulate, transfers were made to agar slants. In many cases pure cultures were secured in this manner, but for most of the identification work single spore cultures were made according to the method described by Hagem (5). This method always gave satisfactory results after a little practise.

For identification, Thom's (17) sucrose potato agar was found most satisfactory as a general medium for all genera, since nearly all of the organisms studied developed and sporulated readily on this medium. The four media described by Thom (17) were used in identifying the members of the genus *Penicillium*, as well as some of the special differentiative media mentioned in his cultural data. Ordinary sterilized bread was found helpful in determining some of the *Mucors*.

As general guides in the identification, Rabenhorst (11) and Engler and Prantl (3) have been followed. Thom's key (17) was used in identifying the members of the genus *Penicillium* Lendner (10) for the *Mucors*, Wehmer (21) for *Aspergillus*, while for other groups the works of Hagem (4), (5), (6), Jensen (7), and Oudemans and Koning (13) have been found very helpful.

The following species were isolated:

<i>Mucor glomerula</i> Lendner (Bainier)	<i>Aspergillus Koningi</i> Oud.
<i>Mucor geophilus</i> Oudemans	<i>Aspergillus glaucus</i> Link
<i>Zygorhynchus Vuilleminii</i> Namys.	<i>Aspergillus nidulans</i> Eidam

<i>Rhizopus nigricans</i> Ehrenberg	<i>Aspergillus clavatus</i> Desmasieres
<i>Penicillium luteum</i> Zukal.	<i>Aspergillus niger</i> van Tiegem
<i>Penicillium commune</i> Thom	<i>Aspergillus</i> sp.
<i>Penicillium expansum</i> Link	<i>Acrostalagmus albus</i> Preuss.
<i>Penicillium pinophilum</i> Hedgcock	<i>Acrostalagmus</i> sp.
<i>Penicillium funiculosum</i> Thom	<i>Cladosporium herbarum</i> Pers.
<i>Penicillium intricatum</i> Thom	<i>Chaetomella horrida</i> Oud.
<i>Penicillium rugulosum</i> Thom (?)	<i>Trichoderma Koningi</i> Oud.
<i>Penicillium decumbens</i> Thom (?)	<i>Verticillium</i> sp.
<i>Aspergillus fumigatus</i> Fres.	<i>Fusarium</i> sp.
<i>Aspergillus flavus</i> Link	<i>Monilia</i> sp.

## PHYSIOLOGICAL STUDIES

The soil employed in the physiological studies was classified as the Carrington loam. In all the tests, 100-gm. portions of sieved, air-dry soil were thoroughly mixed with the organic or inorganic materials in question, placed in 250-cc. Erlenmeyer flasks, made up to the optimum moisture content, and sterilized in the autoclave for 15 minutes at 15 pounds pressure. On cooling, the flasks were inoculated with spore suspensions of pure cultures of the organisms.

For making the spore suspensions, a culture of the organism was grown on Thom's (17) sucrose potato agar for 5 to 7 days. Then 100 cc. of sterile, distilled water were added to the culture flasks, together with a little sterile sand to aid in breaking up the fruiting bodies, and the flasks agitated vigorously for several minutes until a good suspension of spores was secured. The flasks of sterilized soil were then inoculated with 1 cc. of this spore suspension by means of sterile pipettes and the inoculum mixed into the soil with a sterile spatula.

The soils were incubated at 26–28°C., the incubation periods varying with the different series. The moisture content was adjusted to the optimum every 10 days.

*Ammonification*

For determining the ammonifying powers of the organisms, cottonseed meal and dried blood were used as sources of organic matter, 2-gm. portions of each being supplied to the soil in duplicate flasks. Also, 22 cc. of distilled water, plus 2.5 cc. for each gram of organic matter were added to each flask. The flasks were sterilized, inoculated as noted above, incubated for 10 days, and the ammonia present determined by the aeration method (14). The results shown in table I are the averages of duplicate determinations. Where duplicates did not check well the experiment was repeated.

The results show that with few exceptions all the organisms were rather vigorous ammonifiers when either dried blood or cottonseed meal was used. With the single exception of *Acrostalagmus albus*, cottonseed meal proved a better source of nitrogen than dried blood for the fungi, the difference being very marked in some instances. *A. albus* produced a higher percentage of ammonia from dried blood than any other organism (3.57 per cent) and only slightly less from cottonseed meal (33.4 per cent). These results are in accord with those of Coleman (2), who found that cottonseed meal was more readily attacked by fungi than dried blood. McLean and Wilson (12), on the other

hand, obtained contrary results, more  $\text{NH}_3$  being produced from dried blood, while Waksman (19) found a great variation in the ability of different species to attack the two materials, some preferring cottonseed meal and others dried blood. These variations were evident with members of the same genus.

With the exception of *Penicillium commune* and *P. pinophilum*, all of the organisms tested had a relatively high ammonifying power when tested with either dried blood or cottonseed meal. *Mucor glomerula* produced the highest percentage of ammonia from either source, liberating 65.4 per cent of the nitrogen in cottonseed meal, in the form of ammonia.

TABLE 1  
Ammonification by fungi in 10 days

ORGANISM	DRIED BLOOD		COTTONSEED MEAL	
	Increase of $\text{NH}_3\text{-N}$ over check	Per cent ammonified	Increase of $\text{NH}_3\text{-N}$ over check	Per cent ammonified
	mgm.	per cent	mgm.	per cent
<i>Asp. glaucus</i> .....	34.2	12.2	51.8	34.1
<i>Asp. fumigatus</i> .....	42.2	15.1	34.8	22.1
<i>Asp. Koningi</i> .....	47.7	23.2	64.9	43.3
<i>Asp. flavus</i> .....	31.9	10.6	26.2	18.4
<i>Asp. niger</i> .....	8.5	3.0	30.5	27.8
<i>Asp. sp.</i> .....	58.4	20.9	59.4	41.7
<i>Pen. expansum</i> .....	8.0	2.9	32.3	22.7
<i>Pen. commune</i> .....	-1.2	-0.4	6.0	4.2
<i>Pen. pinophilum</i> .....	1.3	0.5	14.1	9.9
<i>Pen. funiculosum</i> .....	55.4	19.8	39.2	27.5
<i>Pen. luteum</i> .....	59.0	20.0	39.8	28.0
<i>Mucor glomerula</i> .....	63.3	22.7	92.9	65.4
<i>Zygorhynchus Vuilleminii</i> .....	40.1	14.3	36.8	25.9
<i>Cladosporium herbarum</i> .....	10.7	3.8	30.9	21.7
<i>Acrostalagmus albus</i> .....	102.1	35.7	47.5	33.4
<i>Verticillium sp.</i> .....	36.7	13.1	66.6	46.8
<i>Fusarium sp.</i> .....	74.7	26.7	64.3	45.2
<i>Monilia sp.</i> .....	94.4	34.9	54.9	38.6

In general, the species belonging to the imperfect genera were more vigorous ammonifiers than members of the genera *Aspergillus* and *Penicillium*. This is in accord with the results of previous workers (12), (19).

#### Phosphorus transformation

In this series, 10 gm. of sieved raw rock phosphate was thoroughly mixed with the air-dry soil, in flasks, 30 cc. distilled water were added, and the flasks were sterilized as noted above. The normal optimum moisture content for this soil was 22 per cent but the addition of the relatively large amount of rock phosphate made necessary a larger addition of water. At the end of the incubation period of 28 days, ammonium-citrate-soluble and water-soluble phosphorus were determined. The results are shown in table 2.

It is evident from the table that no water-soluble phosphorus was set free by the action of the fungi, since in every case the results, whether plus or minus, were so small that they fell within the experimental error. In view of the fact that similar results were obtained in other series the water-soluble determinations were discontinued.

Five organisms, *Aspergillus fumigatus*, *A. flavus*, *Penicillium funiculosum*, *P. luteum*, and *Cladosporium herbarum* appear to have had some action in setting free phosphorus as measured by the citrate-soluble method. This is especially true of *Cladosporium herbarum* and *Penicillium luteum* which liberated 24.9 mgm. and 22.6 mgm., respectively. It is possible that *Aspergillus niger* and *Penicillium pinophilum* which liberated 6.7 mgm. and 8.5

TABLE 2  
Phosphorus transformation by fungi in 28 days

ORGANISM	CITRATE-SOLUBLE P		WATER-SOLUBLE P	
	In culture	Increase over check	In culture	Increase over check
	mgm.	mgm.	mgm.	mgm.
<i>Asp. glaucus</i> .....	27.1	4.9	0.22	-0.22
<i>Asp. fumigatus</i> .....	37.0	14.8	1.25	0.81
<i>Asp. flavus</i> .....	37.4	15.2	0.74	0.30
<i>Asp. Koningi</i> .....	32.3	5.1	2.06	1.62
<i>Asp. niger</i> .....	29.0	6.7	0.65	0.23
<i>Pen. expansum</i> .....	26.0	3.8	0.29	-0.15
<i>Pen. commune</i> .....	22.5	0.2	0.15	-0.29
<i>Pen. pinophilum</i> .....	30.7	8.5	0.15	-0.29
<i>Pen. funiculosum</i> .....	35.1	12.6	0.15	-0.29
<i>Pen. luteum</i> .....	46.1	22.6		
<i>Mucor glomerula</i> .....	26.9	3.4		
<i>Cladosporium herbarum</i> .....	47.1	24.9	1.10	0.66
<i>Acrostalagmus albus</i> .....	21.9	-1.6		
<i>Verticillium sp.</i> .....	23.4	-0.1		
<i>Fusarium sp.</i> .....	19.3	-6.2		
<i>Monilia sp.</i> .....	16.0	-7.5		

mgm., respectively, have had some effect on the phosphate rock, but not enough to be of any importance.

Only one other organism need be mentioned, *Monilia sp.* In this case there was a decrease of 7.5 mgm. below the check, which indicates that the organism utilized in its own metabolism the phosphorus which was set free, with a consequent locking up of phosphorus in the organic form. Further results would be necessary for definite conclusions. It is improbable that enough phosphorus would be locked up in the fungous bodies to have any permanent effect on plant growth, since on decomposition of the mycelium the phosphorus would again become available.

The phosphorus liberated may have been set free either by the action of the carbon dioxide or of the acids produced by the organisms. The ability

to produce rather large quantities of acid, especially oxalic and citric, is generally ascribed to fungi, and under laboratory conditions where relatively small quantities of soil are used, it is quite possible that sufficient acid would be formed to act upon the rock phosphate, rendering a portion of it soluble. From the field standpoint, however, it is probable that the action of carbon dioxide would be more important. Nearly all fungi attack organic matter, decomposing it quite readily, and it is reasonable to believe that the carbon dioxide produced in this decomposition process would have some effect on the insoluble phosphates in the soil. It has been suggested by Kopeloff (8) that fungi may be an important compensating factor in ammonia production in soil where conditions are unfavorable for bacterial growth. It is not improbable that under such conditions they may bear a similar relation to carbon dioxide production, and its resultant effects on inert materials in the soil.

#### *Sulfur oxidation*

In this experiment 2 gm. of flowers of sulfur were added to 100 gm. of soil in flasks, and treated as noted above. Sulfates were determined photometrically at the end of 10 and 20 days. The results are shown in table 3.

It is evident from the table that only one organism tested, *Penicillium luteum*, had any appreciable power of oxidizing sulfur. This organism liberated 17.1 mgm. of sulfate sulfur in 10 days and 20.3 mgm. in 20 days. The experiment was repeated with closely checking results. *Mucor glomerula*, which produced 6.9 mgm. in 20 days seemed to have had a slight action. All of the other results, whether plus or minus, were so small that they fall within the experimental error.

The amount of sulfate sulfur produced by *P. luteum* was one per cent of the application. Inasmuch as this organism has been isolated repeatedly and is one of the most common soil forms, it seems reasonable to conclude that it may be of importance in oxidizing sulfur in the field. It would be interesting to compare this organism with the sulfur-oxidizing bacteria. Where the latter are present in the soil it is probable, however, that their action would overshadow that of the fungus.

Seven strains of *P. luteum* were isolated, all very similar morphologically but quite different physiologically. On sucrose potato agar the colonies ranged in color from pure white in one strain to a brilliant red in another, while the other strains showed various intermediate gradations of color. The reverse of the colonies was also quite variable; some produced a pigment which diffused through the medium while others did not. The morphology of the organisms, however, was so nearly identical that it seems inadvisable to split the group into species, but to retain all of the strains in the "*Penicillium luteum* group." Detailed cultural data are not presented here as the group is being studied further. The strains so far tested were about equal in sulfur-oxidizing power.



TABLE 3  
Sulfur oxidation by fungi in 10 and 20 days

ORGANISM	AFTER 10 DAYS' INCUBATION		AFTER 20 DAYS' INCUBATION	
	SO <sub>4</sub> -S in culture	Increase over check	SO <sub>4</sub> -S in culture	Increase over check
	mgm. of S	mgm.	mgm. of S	
<i>Asp. glaucus</i> .....	5.4	-5.0	6.4	0.1
<i>Asp. fumigatus</i> .....	9.5	-0.9	5.2	1.1
<i>Asp. Koningi</i> .....	12.0	1.6	7.0	0.7
<i>Asp. flavus</i> .....	9.1	-1.3	6.6	0.3
<i>Asp. niger</i> .....	6.2	-4.2	7.3	1.0
<i>Asp. sp.</i> .....	8.2	-2.2	6.1	-0.2
<i>Pen. expansum</i> .....	15.2	4.8	5.9	-0.4
<i>Pen. commune</i> .....	14.6	4.2	8.9	2.3
<i>Pen. pinophilum</i> .....	6.0	-4.4	5.9	-0.4
<i>Pen. funiculosum</i> .....	6.0	-4.4	10.9	4.3
<i>Pen. luteum</i> .....	27.5	17.1	26.6	20.3
<i>Cladosporium herbarum</i> .....	6.8	-3.6	5.8	-0.5
<i>Acrostalagmus albus</i> .....	5.8	-4.6	8.7	2.1
<i>Verticillium sp.</i> .....	6.4	-4.0	5.0	-1.3
<i>Fusarium sp.</i> .....	6.5	-3.9	8.4	2.1
<i>Monilia sp.</i> .....	7.8	-2.6	10.0	3.7
<i>Mucor glomerula</i> .....	14.7	4.3	13.2	6.9

TABLE 4

Transformation of phosphorus by fungi in composts of soil, raw rock phosphate and sulfur

ORGANISM	INCREASE OF CITRATE-SOLUBLE P OVER CHECK			INCREASE OF WATER-SOLUBLE P OVER CHECK	
	After 15 days	After 30 days	After 45 days	After 15 days	After 45 days
	mgm.	mgm.	mgm.	mgm.	mgm.
<i>Asp. glaucus</i> .....	- 2.4	6.5	- 4.8	0.1	-0.7
<i>Asp. fumigatus</i> .....	-11.6	-1.6	- 1.1	0.2	-0.3
<i>Asp. flavus</i> .....	- 6.6	3.7	- 2.9	-0.1	-0.7
<i>Asp. Koningi</i> .....	- 0.1	13.5	17.0	-0.1	-0.7
<i>Asp. niger</i> .....	- 4.9	21.8	- 2.0	0.1	-0.4
<i>Pen. expansum</i> .....	- 7.6	3.9	- 3.0	-0.3	-0.4
<i>Pen. commune</i> .....	- 3.7	-1.2	- 7.9	0.1	-0.0
<i>Pen. pinophilum</i> .....	- 1.4	10.3	-10.8	0.0	-1.0
<i>Pen. funiculosum</i> .....	- 1.2	7.5	10.5	0.2	-1.0
<i>Pen. luteum</i> .....	- 3.4	11.2	3.3		
<i>Mucor glomerula</i> .....	- 9.3	6.8	10.7		
<i>Verticillium sp.</i> .....	- 1.5	2.0	0.5		
<i>Fusarium sp.</i> .....	- 2.2	1.8	3.1		
<i>Monilia sp.</i> .....	- 6.4	19.3	- 0.5		
<i>Cladosporium herbarum</i> .....	- 4.0	4.2	- 8.5		

*Composts of raw rock phosphate and sulfur*

In this experiment 10 gm. of raw rock phosphate and 2 gm. of sulfur were mixed with the soil. Citrate-soluble and water-soluble phosphorus were determined at the end of 15, 30, and 45 days. The results are shown in table 4.

Only three organisms, *Aspergillus Koningi*, *Penicillium funiculosum*, and *Mucor glomerula* showed consistent gains in the amounts of citrate-soluble phosphorus liberated. In each case the amount of soluble phosphorus increased at the end of the successive incubation periods, and the quantities were large enough to indicate some action by the organisms, being 17 mgm. with *A. Koningi*, 10.5 mgm. with *P. funiculosum*, and 10.7 mgm. with *M. glomerula*.

Comparing these results with those on phosphorus transformation where no sulfur was present, it will be noted that neither *Aspergillus Koningi* nor *Mucor glomerula* liberated a measurable quantity of soluble phosphorus in that series. This indicates that the sulfur added in this experiment had a stimulating effect on the ability of the organisms to attack the rock phosphate.

Since none of these three organisms has any measurable sulfur-oxidizing power and consequently would produce little or no sulfuric acid in the soil it is probable that the sulfur caused a stimulation of carbon dioxide production by the fungi, and that this carbon dioxide made more of the phosphorus soluble. The gradual accumulation of soluble phosphorus may be explained either by the fact that the organisms produced relatively large quantities of it or their phosphorus requirements were small.

With very few exceptions the remaining organisms tested showed a decrease under the check at the end of 15 days, a relatively large increase at the end of 30 days, and a decided decrease at the end of 45 days. It will be noted that *Aspergillus niger* and *Monilia sp.* showed appreciable gains in 30 days, while in 45 days they again fell below the check. A possible explanation of these results would be that the soluble phosphorus produced during the first 15 days was utilized by the growing organisms; then, during the next period, as the first "generation" died, the phosphorus was changed to a soluble state through autolysis of the fungous bodies. This period of quiescence was followed by the development of the second "generation" of fungi as the spores germinated, with the consequent utilization of the available phosphorus and a decrease in citrate soluble phosphorus.

As in the previous experiment, the results of the water-soluble determinations were such that no conclusions could be drawn from them.

## DISCUSSION

The results as a whole indicate that fungi have a greater influence on the organic materials in the soil than on the inorganic. With the exception of two species all of the organisms tested were comparatively vigorous in their

action on the organic matter added to the soil, while few exhibited the power of attacking phosphorus and sulfur, and changing to a form available to plants. Many investigators, (5), (9), (16), (19), have shown that fungi are capable of decomposing complex organic materials in the soil, even attacking the complex, inert celluloses and breaking them down to a point where the decomposition may be completed by bacteria.

Undoubtedly fungi are of very great importance in soil as ammonifiers, probably being equal to bacteria in this respect, and available evidence indicates that their principal function in the soil is the decomposition of organic matter. Their effect on the mineral constituents appears to be more limited. Eight organisms showed the power of rendering a portion of the phosphorus in raw rock phosphate soluble, while only one was capable of oxidizing elemental sulfur. It cannot be stated, however, that fungi are of no importance in making such materials available under field conditions, because most of the common soil forms are active decomposers of organic matter, and they occur in such numbers that sufficient carbon dioxide would probably accumulate from the decomposition processes to act upon the complex mineral compounds. They would be especially important where conditions are such that bacterial growth is depressed.

Another fact which must be kept in mind is that in these experiments only pure cultures of fungi were used. It is the usual experience in microbiological work that mixed cultures, which more nearly represent natural conditions, give higher results than pure cultures, and it is quite probable that such would be the case here.

#### SUMMARY

1. In these experiments 28 species of fungi, representing 12 genera, were isolated from 5 soils which varied in lime requirement from zero to 5 tons per acre. The organisms isolated include the common soil genera, but represent a small proportion of the total soil flora.

2. Waksman and Fred's synthetic acid medium proved the most satisfactory for isolating fungi, since bacterial development is depressed on this medium.

3. With two exceptions, the organisms tested were rather vigorous ammonifiers. The members of the imperfect genera exhibited the greatest ammonifying ability while members of the genus *Penicillium* were the weakest.

4. All of the species tested ammonified cottonseed meal more readily than dried blood, with the single exception of *Acrostalagmus albus*.

5. Five species, *Aspergillus fumigatus*, *A. flavus*, *Penicillium funiculosum*, *P. luteum*, and *Cladosporium herbarum* were shown to have the ability of liberating soluble phosphorus from raw rock phosphate, as measured by the ammonium citrate method.

6. *Penicillium luteum* was the only organism tested which had the power of oxidizing free sulfur to the sulfate form. Seven strains of this organism were isolated, and all showed about the same sulfur-oxidizing power.

7. With composts of sulfur and raw rock phosphate, only three organisms, *Aspergillus Konigi*, *Penicillium funiculosum*, and *Mucor glomerula* showed consistent gains in the amount of citrate soluble phosphorus liberated through successive incubation periods of 15 days. With few exceptions the remaining species showed a decrease under the check in 15 days, an increase in 30 days, followed by a marked decrease in 45 days. It is probable that these variations correspond with the development of successive generations of the organisms.

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